The evolution of attachment structures in two megadiverse insect lineages
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Acercaria and Diptera

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1 Introduction

Many insects are capable of climbing and walking upside down on diverse substrates using adhesive organs on their legs (Scherge & Gorb, 2001). This ability has fascinated researchers for more than a century (Dewitz, 1884; Knoll, 1914; Nachtigall, 1974; Stork, 1983; Beutel & Gorb, 2006). The attachment devices making this possible are the main topic of this thesis. The evolution of attachment structures is likely closely related to the evolution of wings (e.g.,, Beutel & Gorb, 2001). Flying made it necessary to be able to land and walk on a broad variety of objects and substrates, especially on plant surfaces.

Despite the enormous diversity of insects, only two main alternative designs of tarsal adhesive organs have evolved. ‚Hairy' pads are densely covered with flexible microtrichia and occur in several insect orders, including flies, beetles and webspinners (Beutel & Gorb, 2001). ‚Smooth' adhesive pads have a relatively even surface profile and a specialized, soft and very flexible cuticle. They are present in many insects including ants, bees, cockroaches and stick insects (Beutel & Gorb, 2001, 2006; Scholz et al., 2008). In both systems adhesion is mainly realized by a fluid that is secreted into the contact zone (Gorb, 2001). Both systems are flexible and can adapt to different surfaces. However, both are prone to abrasion when used on rough surfaces.

This thesis focuses on the intricate relationship between attachment structures and plant surfaces. The surfaces of many plants are covered with trichomes or a waxy layer, which makes attachment and locomotion difficult for most insects (e.g., Barthlott et al., 1998). Especially the wax platelets, which cover many plant surfaces, reduce the attachment ability of insects dramatically by contaminating the adhesive devices (e.g., Gorb et al., 2005; Gorb et al., 2008). However, there seems to be a "self-cleaning" mechanism (Clemente et al., 2010), where the insects loose the waxes step by step without having to groom their tarsi.

Since plants are often not only a food source, but also the habitat and mating site for many herbivorous insects, the ability to attach and walk efficiently on different surface types may play a role in host fidelity and specialization, maybe even in the context of speciation. In some insects (e.g., oak-feeding aphids) the ability to attach successfully to the plant surface has likely contributed to the specialization on the specific plant substratum (Kennedy, 1986). It is conceivable that a similar specialization related to attachment and locomotion may be found in other herbivorous insects as well. The thesis investigates this possibility using the example of the pea aphid complex. The pea aphid (*Acyrthosiphon pisum*), a legume specialist, encompasses at least 11 genetically distinct sympatric varieties (=host races; e.g., Via, 2001; Peccoud & Simon, 2010). Each host race shows a preference for a certain legume species. The wax coverage of the plants differs immensely. Using traction force measurements it is

analysed if there is a difference in the attachment ability of the host races on host and non-host plants with very different surface topologies.

Another main focus of this thesis is the evolution of attachment devices in two very successful lineages of insects. On the one hand Acercaria (true bugs, cicada, aphids, plant lice etc.) which mainly exhibit smooth attachment structures, and on the other hand Diptera (flies and gnats), in which hairy devices prevail. Both lineages display an unusually broad variation of different types of attachment devices such as arolium, pulvilli, euplantulae, claw pads and hairy tarsal soles.

One major aim was a detailed documentation of the adhesive devices with modern morphological approaches in both lineages.

Acercaria was introduced by Börner (1904) for a hemimetabolous lineage comprising "Psocoptera" (bark lice), Phthiraptera (true lice), Thysanoptera (thrips), and Hemiptera (bugs). Hennig (1969) considered Zoraptera (ground lice, angel insects) as its sister taxon and referred to the more inclusive lineage as Paraneoptera. With more than 100,000 described species, Acercaria are an extremely diverse and successful lineage of insects. The group is characterized by a very broad spectrum of feeding habits. It comprises detritivores (mainly "Psocoptera"), highly specialized ectoparasites (Phthiraptera), miniaturized forms feeding on fungi or algae (Phlaeothripidae), numerous species feeding on plant saps (major part of Thysanoptera and Hemiptera), and also predators (several lineages of Heteroptera). Blood feeding on vertebrates evolved twice in Acercaria: once in Phthiraptera (Anoplura and Rhynchophthirina) and at least three times in Heteroptera [Cimicidae, Reduviidae, and Rhyparochromidae (Schuh & Slater, 1995)]. Acercaria are also extremely versatile in their habitat choices. Numerous species move efficiently on plant surfaces (e.g., Gorb et al., 2001) but there are also highly specialized semiaquatic and aquatic lineages (Gerromorpha, Nepomorpha), groups specialized on leaf litter and soil or subcortical habitats, and ectoparasites adapted to feathers or hairs of their warm-blooded vertebrate hosts (e.g., Weber, 1969; Grimaldi & Engel, 2005). With the necessity to cope with a broad spectrum of different surfaces, a tremendous variety of attachment devices has evolved in Acercaria.

Information on the tarsal and pretarsal morphology of Acercaria is scattered in the literature. Within the group, the structure and function of attachment devices have been investigated in detail for a few selected species, such as the planthopper *Lycorma delicatula* (Frantsevich et al., 2008), several aphids (e.g., Carver & White, 1971; Lees & Hardie, 1988; Dixon et al., 1990), the true bug *Pameridea roridulae* (Voigt & Gorb, 2008), and the head louse *Pediculus humanus* (Soler-Cruz & Martin-Mateo, 2009). Systematic investigations of the pretarsal and tibial structures of Reduviidae and Miridae (Heteroptera) have been carried out by Weirauch (2005, 2007) and Schuh (1976), respectively.

So far, adhesive devices of representatives of phylogenetically important ("basal") acercarian subgroups have received little attention. A comprehensive comparative study of tarsal and pretarsal structures of all major lineages was missing. Especially the subgroups of Sternorrhyncha and Psocoptera have been largely neglected. In most available studies only one representative of one of the four lineages of Sternorrhyncha was included, even though these groups are morphologically highly heterogeneous.

The monophyly of Acercaria appears to be well supported by morphological characters (Hennig, 1969; Kristensen, 1981; Kristensen et al., 1991; Beutel & Gorb, 2001, 2006), even though it is occasionally rejected by molecular data (e.g., Yoshizawa & Johnson, 2005; Ishiwata et al., 2011).

The monophyly of the major subgroups Psocodea (parasitic Phthiraptera and free-living "Psocoptera"; Seeger, 1975; Rudolph & Knülle, 1982; Lyal, 1985) and Hemiptera (e.g., Kristensen, 1981; Cryan & Urban, 2012) is also well supported.

Despite considerable recent progress in the phylogenetic investigation of Acercaria (e.g., Yoshizawa & Johnson, 2003; Johnson et al., 2004; Cryan & Urban, 2012), a phylogenetic study covering all major lineages and a broad spectrum of morphological characters was still wanting. However, for developing an evolutionary scenario for attachment devices a well-founded phylogenetic hypothesis is vital. In one part of this thesis the relationships are addressed using morphological data including attachment structures. Unpublished results from a recent phylogenomic project (1KITE: 1000 Insect Transcriptome Evolution) are also used to evaluate the evolution of attachment devices in Acercaria.

The Diptera are another extremely successful lineage, with an immense economic impact and very likely the insect group with the greatest negative effect on human health (e.g., Beutel et al., 2014). Diptera contain approximately 154,000 described species and are one of the extremely species-rich groups of Holometabola (Grimaldi & Engel, 2005). The monophyly has never been questioned and is supported by convincing apomorphies such as the metathoracic halteres and the labial palpi transformed into labellae (Hennig, 1973). Dipterans occur on all continents including Antarctica and in all zoogeographic regions. Traditionally Diptera is divided into "Nematocera" and Brachycera (e.g., Hennig, 1953). The former are a paraphyletic grade containing all non-brachyceran groups (Hennig, 1973). The neutral term "lower Diptera" is used in more recent contributions (e.g., Yeates et al., 2007; Wiegmann et al., 2011; Lambkin et al., 2013). In contrast, the monophyly of Brachycera is well supported and undisputed (Hennig 1973; Woodley 1989; Yeates et al., 2007).

The remarkable diversification (Wiegmann et al., 2011) was accompanied by the evolution of different kinds of attachment structures. Functional principles (Niederegger & Gorb,

2003) and the morphology of attachment pads have been described for different members of Brachycera (e.g., Gorb, 1998; Bauchhenß & Renner, 1977; Walker, 1985). However, surprisingly, the adhesive devices of lower Diptera ("Nematocera") have been greatly neglected so far. Although characters of pretarsal structures were included in several studies (e.g., Oosterbroeck & Courtney, 1995; Sinclair & Cumming, 2006) they were usually not illustrated.

Four main issues are addressed in the thesis:

1. Morphology of attachment structures

A detailed description of the morphology and ultrastructure of attachment devices is one of the major aims of the present thesis. In total 77 (incl. 18 outgroups) species are studied using a broad array of different techniques, with emphasis on high quality documentation (Studies I, II, III).

2. Phylogeny of Acercaria

Another aim of this thesis is the reconstruction of the phylogenetic relationships of the acercarian suborders. A character set which allows a formal analysis of acercarian relationships independently of molecular data is provided. Combined with original studies a morphological data matrix of 118 characters of all body parts for 25 acercarian species is presented, evaluated and discussed (Study I).

3. Evolutionary scenarios

Based on the phylogenetic results, scenarios for the evolution of the acercarian attachment devices are discussed. Character evolution of pretarsal structures in Diptera was evaluated by mapping the observed character states onto an already existing phylogeny (Wiegmann et al. 2011, fig. 1). The morphological changes that single attachment devices (arolia) underwent are discussed as well (Study III).

4. Interactions of plant surfaces and attachment devices

The possible role the attachment ability might play in speciation is investigated in the pea aphid complex. Using traction force measurements it is tested how the different host specific varieties of pea aphids perform on host and non-host plants with very different surface topologies (Study IV). It is shown how the waxy surface of some plants reduces the attachment ability.

2 Material and techniques

2.1 Material examined

In the present study 27 members of Acercaria (plus 8 outgroup taxa) and 32 members of Diptera (plus 10 outgroup taxa within the Holometabola) were investigated (Table 1).

2.2 Light microscopy and image editing

External features were examined and drawn using a stereo microscope MZ 12.5 with a camera lucida (LEICA). Line drawings were further processed with Adobe Illustrator®. All figures were assembled in Adobe Photoshop® and labelled with Adobe Illustrator® (San Jose, California, USA).

2.3 Scanning electron microscopy (SEM)

For scanning electron microscopy (Philips XL30 ESEM) specimens were dehydrated with ethanol in several steps, dried either at the critical point (Emitech K850) or using HMDS (hexamethyldisilazane; Brown, 1993), sputter-coated with gold (Emitech K500), and fixed on a rotatable specimen holder (Pohl, 2010). Scandium software (Soft Imaging System, Münster, Germany) was used for obtaining high resolution images. To investigate the ultrastructure of arolia specimens were embedded in methacrylate. Cross and longitudinal sectioning was carried out so that the middle of the arolium was cut. Methacrylate was then dissolved using Xylol. Xylol was replaced gradually by acetone and specimens were dried at the critical point and subsequently examined under the scanning electron microscope.

2.4 Histology

Specimens were dehydrated in an ethanol series and embedded in Araldite CY 212R, sectioned at 1 µm with a microtome (Microm HM360) equipped with a diamond knife and stained with Toluidin blue (Waldeck GmbH & Co., KG/Division Chroma, Münster, Germany). Subsequently the sections were photographed using a Zeiss Axioskop with a Pixellink PL686CO digital camera and aligned with Amira 4.1.2 (Mercury Computer Systems, Berlin, Germany).

2.5 Computer based 3-dimensional reconstruction

Reconstructions were based on serial sections. Segmentation was performed manually with Visage Imaging® Amira 4.1.2 by labeling each discrete structure. Autodesk® Maya 2013 was used for final surface polishing, smoothing and rendering.

Table 1: Taxon sampling and source of data for specimens not examined in the thesis.

Taxon	**Species**	**Studied in the PhD project**	**Literature source**
Odonata	*Ictinus angulosus* Selys, 1854		Beutel & Gorb 2001
Plecoptera	*Nemoura cinerea* Latreille, 1796		Beutel & Gorb 2001
Orthoptera	*Locusta migratoria* (L., 1758)	x	
	Tettigonia viridissima (L., 1758)	x	
Zoraptera	*Zorotypus weidneri* New, 1978	x	
Psocoptera	*Caecilius flavidus* (Stephens, 1830)	x	
	Cerobasis sp.	x	
	Embidiopsocus sp.	x	
	Liposcelis sp.	x	
Phthiraptera	*Columbicola* sp.	x	
	Haematomyzus elephantis (Piaget, 1869)	x	
	Pediculus humanus capitis L., 1758		Soler-Cruz & Martin-Mateo 2009
	Pediculus humaus corporis L., 1758	x	
	Phthirus pubis L., 1758.	x	
	Trichodectes melis (Fabricius, 1805)	x	
	Trinoton anserinum (Fabricius, 1805)	x	
Thysanoptera	*Frankliniella* sp.	x	
Sternorrhyncha	*Acyrthosiphon pisum*	x	
	Aleyrodes sp.	x	
	Cacopsylla sp.	x	
	Coccus sp.	x	
	Drepanosiphum plantanoides (Schrank, 1801)	x	
	Microsiphum sp.	x	
	Pseudococcus sp.	x	
Coleorrhyncha	*Hackeriella veitchi* (Hacker, 1932)	x	
Heteroptera	*Ceratocombus australiensis* Gross, 1950	x	
	Corixidea sp.	x	
	Graphosoma lineatum (L., 1758)	x	
	Pentatoma rufipes (L., 1758)	x	
	Systelloderes sp.	x	
Auchenorrhyncha	*Centrotus cornutus* (L., 1758)	x	
	Cercopis vulnerata (Rossi, 1807)	x	
	Cicadella viridis (L., 1758)	x	
	Cicadetta montana Scopoli 1772	x	
	Cixius sp.	x	

Taxon	Species	Studied in the PhD project	Literature source
Auchenorrhyncha	*Dictyophora europaea* (L. 1767)	x	
	Gargara genistae (Fabricius, 1775)	x	
	Javasella sp.	x	
	Ledra aurita L., 1758	x	
	Neophilaenus sp.	x	
	Philaenus spumarius (L., 1758)	x	
	Strictocephala bisonia Kopp & Yonke, 1977	x	
Hymenoptera	*Xyela* sp.		Beutel & Gorb 2001
Neuroptera	*Chrysopa perla* (L., 1758)		Beutel & Gorb 2001
	Nevrorthus sp.	x	
Strepsiptera	*Mengenilla chobauti* (Hofeneder, 1910)		Pohl & Beutel 2004
Lepidoptera	*Micropterix* sp.	x	
Mecoptera	*Boreus westwoodi* Hagen, 1866	x	
	Caurinus dectes Russell, 1979	x	
	Hylobittacus apicalis (Hagen, 1861)	x	
	Merope tuber Newman, 1838	x	
	Nannochorista sp.	x	
	Panorpa communis L., 1758	x	
Siphonaptera	*Ctenocephalus felis* (Bouché, 1835)	x	
Diptera	*Androprosopa* sp.	x	
	Anopheles maculipennis (Meigen, 1818)	x	
	Antocha sp.	x	
	Axymyia furcata McAtee, 1921	x	
	Bibio marci L., 1758	x	
	Ceratopogonidae, n.n.	x	
	Chaoborus christallinus (De Geer, 1776)	x	
	Chironomidae, n.n.	x	
	Coboldia fuscipes (Meigen, 1830)	x	
	Corethrella appendiculata Grabham, 1906	x	
	Cylindrotoma distictissima (Meigen, 1818)	x	
	Deuterophlebia coloradensis Pennak, 1945	x	
	Drosophila melanogaster Meigen, 1830	x	
	Edwardsina gracilis Edwards, 1929	x	
	Episyrphus balteatus (De Geer, 1776)	x	
	Exechia separata Lundstrom, 1921	x	
	Glossina palpalis gambiensis (Vanderplank, 1911)	x	
	Macrocera centralis Meigen, 1818	x	
	Mayetiola destructor (Say, 1817)	x	

Taxon	Species	Studied in the Phd project	Literature source
Diptera	*Mischoderus* sp.	x	
	Nymphomyia dolichopeza Courtney, 1994	x	
	Pachygaster atra (Panzer, 1798)	x	
	Pedicia rivosa L., 1758	x	
	Psychoda alternata Say, 1824	x	
	Ptychoptera contaminata L., 1758	x	
	Simulium sp.	x	
	Spathobdella falcifera (Lengersdorf, 1933)	x	
	Stilpnogaster aemula (Meigen, 1920)	x	
	Sylvicola fenestralis (Scopoli, 1763)	x	
	Tabanus sp.	x	
	Tipula maxima Poda, 1761	x	
	Trichocera fuscata Meigen, 1818	x	

2.6 Cladistic analysis

Winclada 1.00.08 (Nixon, 1999) was used to enter the data in a matrix, and NONA (Goloboff, 1999) and TNT (Goloboff et al., 2008) for calculating minimum length trees (Ratchet, search settings: 1000 replicates, characters nonadditive, nonweighted). Branch support values (Bremer, 1994) were calculated with the function implemented in TNT. To evaluate the character evolution of pretarsal structures in Diptera observed character states were mapped onto the cladogram of Wiegmann et al. (2011, fig. 1) using the function implemented in Mesquite (trace characters over trees, reconstruction method parsimony ancestral states; Maddison & Maddison, 2011).

2.7 Traction force measurements

Traction experiments with tethered walking aphids were carried out to measure insect attachment forces on different surfaces. Force tests were performed with a load cell force transducer (10 g capacity, Biopac Systems Ltd., Santa Barbara, CA, USA). The aphids were attached to the force sensor with a hair (10–15 cm long) glued to the dorsal surface of the insect with a droplet of water soluble glue. Leaflets were cut off the plant and attached with double-sided adhesive tape to a horizontal glass plate. Five types of substrates were tested: (1) abaxial leaflet surface of *Pisum sativum*, (2) abaxial leaflex surface of *Vicia faba*, (3) abaxial leaflet surface of *Trifolium pratense*, (4) abaxial leaflet surface of *Medicago sativa* and (5) a glass plate as a control. Nine force tests were carried out with each individual aphid: first on the glass substrate, then on one of the plant surfaces, again on the glass surface, on another plant surface, again on the glass and so on. The force generated by the insect walking horizontally on test substrates was measured. For each plant surface type, experiments with 16 individual insects were conducted. In all, 48 insects were tested and 432 force measurements were performed.

2.8 Terminology and Glossary

Unless indicated otherwise the morphological terminology follows Dashman (1953) and Beutel & Gorb (2001). Acercaria is used for the monophyletic clade comprising only Hemiptera, Thysanoptera and Psocodea whereas Zoraptera is also included in Paraneoptera.

Glossary:

Acanthae: projections of the cuticle of unicellular composition with no sockets or sense cells.

Arcus: U-shaped band, which embraces the base of the arolium ventrally with its arms

extending distally in the lateral walls on either side.

Arolium: the median lobe between the claws of the pretarsus. It can be completely membranous or partly sclerotized.

Auxiliae: lateral sclerites beneath the bases of the claws (=Basipulvilli s. Dashman, 1953).

Empodium: a median process between the pulvilli. It arises from the distal end of the unguitractor plate, is spine-shaped or lobe-like, and is often similar in shape to the pulvilli.

Euplantulae: flexible, pad-like structures without hairs, situated on the ventral side of one or more tarsomeres.

Manubrium: elongate medial sclerite in the dorsobasal region of the arolium. It is articulated proximally on the end of the tarsus between the bases of the claws. By its narrowed distal end it is attached like a handle to the base of the arolium.

Parempodia: bristle-like or fleshy appendages of the distal part of the unguitractor plate.

Planta: distal part of an unguitractor plate, which is divided into two sclerites.

Pretarsus: the apical part of the hexapod leg, closely associated with the distal end of the terminal tarsomere.

Pulvilli: smooth or hairy paired lateral membranous lobes ventral to the claws. They are located on the auxiliae, which participate in control of pulvilli movements.

Unguitractor plate: median basal plate of the ventral surface of the pretarsus. It is usually invaginated into the end of the terminal tarsomere. Its surface is highly variable.

3 Published results

Study I: **Friedemann K**, Spangenberg R, Yoshizawa K, Beutel RG (2014)
Evolution of attachment structures in the highly diverse Acercaria (Hexapoda)
Cladistics 30, 170-201

Study II: **Friedemann K**, Beutel RG (in press)
Morphology of arolia in Auchenorrhyncha (Insecta, Hemiptera)
Journal of Morphology, DOI 10.1002/jmor.20290

Study III: **Friedemann K**, Schneeberg K, Beutel RG (2014)
Fly on the wall – attachment structures in lower Diptera
Systematic Entomology 39, 460-473

Study IV: **Friedemann K**, Kunert G, Gorb E, Beutel RG (in prep.)
Attachment forces of pea aphid host races on different legume species
Ecological Entomology

3.1 Study I

Friedemann K, Spangenberg R, Yoshizawa K, Beutel RG (2014)
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Abstract: Acercaria display an unusually broad array of adhesive devices occurring on different parts of the legs. Attachment structures of all major subgroups are described and illustrated. Nineteen characters of the distal leg region were combined with a data matrix containing 99 additional morphological characters of different body parts. The results of the cladistic analysis are largely congruent with current hypotheses. Zoraptera are not retrieved as close relatives of Acercaria. The monophyly of the entire lineage and of the major subgroups Psocodea, Phthiraptera, and Hemiptera is confirmed. Our data also support the monophyly of Auchenorrhyncha and a sister-group relationship between Thysanoptera and Hemiptera (Condylognatha). In contrast to other lineages of insects, the hairy type of adhesive device is present only in one group within the Acercaria (Heteroptera, Cimicomorpha). The arolium is present in the groundplan but missing in several groups (e.g. Psocodea, Cicadoidea, Aphidoidea). Pretarsal pulvilli evolved several times independently. Tarsal euplantulae and different specialized clasping devices have evolved within Phthiraptera, whereas pretarsal attachment devices are missing in this ectoparasitic group. The potential to modify pretarsal attachment devices in their structural details has probably contributed to the very successful diversification of the predominantly phytophagous Hemiptera.

Significance in the present thesis: This study is focused on the evolution of attachment structures in Acercaria. The phylogeny of this lineage was reconstructed with a morphological datamatrix containing 99 characters. The pretarsal structures of 26 species were investigated. Based on the obtained phylogeny the evolution of those structures was discussed.

Own contribution: 80%

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Evolution of attachment structures in the highly diverse Acercaria (Hexapoda)

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Abstract

Acercaria display an unusually broad array of adhesive devices occurring on different parts of the legs. Attachment structures of all major subgroups are described and illustrated. Nineteen characters of the distal leg region were combined with a data matrix containing 99 additional morphological characters of different body parts. The results of the cladistic analysis are largely congruent with current hypotheses. Zoraptera are not retrieved as close relatives of Acercaria. The monophyly of the entire lineage and of the major subgroups Psocodea, Phthiraptera, and Hemiptera is confirmed. Our data also support the monophyly of Auchenorrhycha and a sister-group relationship between Thysanoptera and Hemiptera (Condylognatha). In contrast to other lineages of insects, the hairy type of adhesive device is present only in one group within the Acercaria (Heteroptera, Cimicomorpha). The arolium is present in the groundplan but missing in several groups (e.g. Psocodea, Cicadoidea, Aphidoidea). Pretarsal pulvilli evolved several times independently. Tarsal euplantulae and different specialized clasping devices have evolved within Phthiraptera, whereas pretarsal attachment devices are missing in this ectoparasitic group. The potential to modify pretarsal attachment devices in their structural details has probably contributed to the very successful diversification of the predominantly phytophagous Hemiptera.

Introduction

Acercaria was introduced by Börner (1904) for a hemimetabolous lineage comprising "Psocoptera" (bark lice), Phthiraptera (true lice), Thysanoptera (thrips), and Hemiptera (bugs). Hennig (1969) considered Zoraptera (ground lice, angel insects) as its sister taxon and referred to the more inclusive lineage as Paraneoptera. Today, polyneopteran affinities of Zoraptera become more and more evident (e.g. Wheeler et al., 2001; Blanke et al., 2012; see also Trautwein et al., 2012); and Aceraria (e.g. Börner, 1904; Seeger, 1975; Kristensen, 1981) and Paraneoptera (e.g. Yoshizawa and Saigusa, 2001; Grimaldi and Engel, 2005) are used by different authors for the "hemipteroid assemblage". To avoid confusion we consistently use the former term, which is also less ambiguous.

With more than 100 000 described species, Acercaria are an extremely diverse and successful lineage of insects. The group is characterized by a very broad spectrum of feeding habits. It comprises detritivores (mainly "Psocoptera"), highly specialized ectoparasites (Phthiraptera), miniaturized forms feeding on fungi or algae (Phlaeothripidae), numerous species feeding on plant saps (major part of Thysanoptera and Hemiptera), and also predators (which are restricted to several lineages of Heteroptera). Blood feeding on vertebrates evolved twice in Acercaria: once in Phthiraptera (Anoplura and Rhynchophthirina) and at least three times in Heteroptera [Cimicidae, Reduviidae, and Rhyparochromidae (Schuh and Slater, 1995)]. Acercaria are also extremely versatile in their habitat choices. Numerous species move efficiently on plant surfaces (e.g. Gorb et al., 2001) but there are also highly specialized semiaquatic and aquatic lineages (Gerromorpha, Nepomorpha), groups specialized on leaf litter and soil or

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subcortical habitats, and ectoparasites adapted to feathers or hairs of their warm-blooded vertebrate hosts (e.g. Weber, 1969; Grimaldi and Engel, 2005).

With the necessity to cope with a broad spectrum of different surfaces, a tremendous variety of attachment devices has evolved in Acercaria. Within the group, the structure and function of attachment devices have been investigated in detail for a few selected species, such as the planthopper *Lycorma delicatula* (Frantsevich et al., 2008), several aphids (e.g. Carver and White, 1971; Lees and Hardie, 1988; Dixon et al., 1990), the true bug *Pameridea roridulae* (Voigt and Gorb, 2008), and the head louse *Pediculus humanus* (Soler-Cruz and Martin-Mateo, 2009). Systematic investigations of the pretarsal and tibial structures of Reduviidae and Miridae (Heteroptera) have been carried out by Weirauch (2005, 2007) and Schuh (1976), respectively. For a summary of cimicomorphan attachment devices see Schuh et al. (2009).

So far, adhesive devices of basal representatives of the subgroups of Acercaria have received little attention. A comprehensive comparative study of pretarsal structures of all major lineages is still missing. Especially the subgroups of Sternorrhyncha and Psocoptera have been largely neglected. In most available studies only one representative of one of the four lineages of Sternorrhyncha was included, even though these groups are morphologically highly heterogeneous.

Information on the tarsal and pretarsal morphology of Acercaria is scattered in the literature. An additional problem is the inconsistent nomenclature. Some authors refer to any kind of pretarsal attachment structure as "arolium". For *Lygus hesperus* (Miridae), for instance, Shrestha et al. (2007) disregards the commonly used nomenclature (see e.g. Beutel and Gorb, 2001) and refers to the attachment structures as arolia. However, his figures show clearly that these paired structures are in fact pulvilli. Another inappropriate term that persists is "dorsal arolium" (e.g. Cobben, 1978; Schuh and Slater, 1995; Schuh and Polhemus, 2009) even though this structure is clearly not an attachment device, but a peg-like or trichoform structure, and most likely a sensillum (= dorsomedian sensillum after Weirauch, 2005).

The monophyly of Acercaria appears to be well supported by morphological characters (Hennig, 1969; Kristensen, 1981; Kristensen et al., 1991; Beutel and Gorb, 2001, 2006), even though it is frequently rejected by molecular data (e.g. Yoshizawa and Johnson, 2005; Ishiwata et al., 2011). The monophyly of the major subgroups Psocodea (parasitic Phthiraptera and free-living "Psocoptera"; Seeger, 1975; Rudolph and Knülle, 1982; Lyal, 1985) and Hemiptera (e.g. Kristensen, 1981; Cryan and Urban, 2012) is also well supported. A sister-group relationship between Liposcelididae and the true lice also appears well established based on morphological (Lyal, 1985) and molecular data (Yoshizawa and Johnson, 2003; Johnson et al., 2004). However, what is still disputed is the monophyly of the true lice (Amblycera, Ischnocera, Rhynchophthirina, and Anoplura) (Barker et al., 2003; small-subunit rDNA; Johnson et al., 2004; 18S rDNA), the placement of Thysanoptera (Condylognatha versus Micracercaria, e.g. Yoshizawa and Saigusa, 2001; see also Willmann and Dathe, 2005; Grimaldi and Engel, 2005), the interrelationships of the hemipteran subgroups, and the monophyly of Auchenorrhyncha (e.g. Campbell et al., 1995; Cryan and Urban, 2012).

Despite considerable recent progress in the phylogenetic investigation of Acercaria (e.g. Yoshizawa and Johnson, 2003; Johnson et al., 2004; Cryan and Urban, 2012), a phylogenetic study covering all major lineages and a broad spectrum of morphological characters was still wanting. It is one aim of this study to provide a character set allowing a formal analysis of acercarian relationships, independently of molecular data that are already available [e.g. Johnson et al., 2004 (focused on Phthiraptera); Cryan and Urban, 2012 (focused on Hemiptera)] or will be available in the near future (see the Acercaria subproject of 1KITE: www.1KITE.org). However, the main focus is on the evolution of attachment structures in Acercaria. Our goal is to describe and document the attachment devices of representatives of all the major lineages, and to develop an evolutionary scenario for the relevant structures based on a cladistic analysis of characters of all body parts (see Appendix 1). Taxa were chosen for their (presumably) basal phylogenetic position. All figures show the attachment structures of females. Future phylogenetic analyses based on extensive molecular data (transcriptomes; see www.1KITE.org) will provide a robust basis for testing the hypotheses presented in this study.

Methods

Scanning electron microscopy

For SEM (Philips XL30 ESEM; Fei, Eindhoven, The Netherlands), specimens were completely dehydrated with ethanol (100%) over several stages, dried at the critical point (Emitech K850; Emitech, Ashford, Kent, UK) or treated with HMDS (hexamethyldisilazane; Brown, 1993), sputter-coated with gold (Emitech K500; Emitech), and fixed on a rotatable specimen holder (Pohl, 2010). Scandium software (Soft Imaging System, Münster, Germany) was used to obtain high-resolution images.

Cladistic analysis

We analysed 118 characters of the head, thorax, abdomen, and attachment structures (of the midlegs)

of 25 representatives of Acercaria plus eight outgroup taxa. Winclada 1.00.08 (Nixon, 1999) was used to enter the data in a matrix, and NONA (Goloboff, 1999) and TNT (Goloboff et al., 2008) for calculating minimum length trees (Ratchet, search settings: 1000 replicates, characters nonadditive, nonweighted). Branch support values (Bremer, 1994) were calculated with the function implemented in TNT.

Glossary

The terms used here are in accordance with the definitions of Dashman (1953) and Beutel and Gorb (2001).

Arcus: elastic, U-shaped band that embraces the base of the arolium ventrally with its arms extending distally in the lateral walls on either side.

Arolium: median lobe between the claws of the pretarsus. It can be completely membraneous or at least partly sclerotized.

Auxiliae: lateral sclerites beneath the bases of the claws.

Claws: hollow, multicellular, movable structures that articulate dorsally at the distal end of the tarsus.

Empodium: median process between the pulvilli that arises from the distal end of the unguitractor plate, is spine-shaped or lobe-like, and is often similar in form to the pulvilli.

Euplantulae: flexible, pad-like structures without hairs on the ventral side of one or more tarsomeres.

Manubrium: elongate medial sclerite in the dorsobasal region of the arolium. It is articulated proximally on the end of the tarsus between the bases of the claws and by its narrowed distal end it is attached like a handle to the base of the arolium.

Parempodia: bristle-like or fleshy appendages of the distal part of the unguitractor plate.

Planta: distal part of an unguitractor plate, which is divided into two sclerites.

Pretarsus: terminal part of the hexapod leg, closely associated with the distal end of the terminal tarsomere.

Pulvilli: smooth or hairy paired lateral membraneous lobes ventral to the claws. They are located on the auxiliae, which participate in control of pulvillar movements.

Tarsus: distal part of a hexapod leg.

Unguitractor plate: median basal plate of the ventral surface of the pretarsus, to which the unguitractor apodeme is attached. It is usually invaginated into the end of the terminal tarsomere. Its surface is highly variable.

Taxa examined

Auchenorrhyncha.

Cicadomorpha: Cercopidae, *Philaenus spumarius* (L., 1758), *Cercopis vulnerata* (Rossi, 1807).

Cicadoidea: Cicadidae, *Cicadetta montana* Scopoli 1772.

Membracoidea: Membracidae, *Centrotus cornutus* (L., 1758). Cicadellidae, *Cicadella viridis* (L., 1758).

Fulgoromorpha: Cixiidae, *Cixius* sp., Delphacidae, *Javasella* sp., *Dictyophora europaea* (L. 1767).

Coleorrhyncha. Peloridiidae, *Hackeriella veitchi* (Hacker 1932).

Heteroptera.

Dipsocoromorpha: Dipsocoridae, *Ceratocombus australiensis* Gross, 1950, Schizopteridae, *Corixidea* sp.

Enicocephalomorpha: Enicocephalidae, *Systelloderes* sp.

Pentatomomorpha: Pentatomidae, *Graphosoma lineatum* (L., 1758), *Pentatoma rufipes* (L., 1758).

Psocodea

Phthiraptera.

Amblycera: Menoponidae, *Trinoton anserinum* [Fabricius (J.C.), 1805].

Anoplura: Pediculidae, *Pediculus humanus capitis* L., 1758, *Pediculus humaus corporis* L., 1758; *Phthirus pubis* L., 1758.

Ischnocera: Trichodectidae, *Trichodectes melis* [Fabricius (J.C.), 1805]; Philopteridae, *Columbicola* sp.

Rhynchophthirina: Haematomyzidae, *Haematomyzus elephantis* (Piaget, 1869).

Psocoptera.

Psocomorpha: Caeciliidae, *Caecilius flavidus* (Stephens, 1830).

Troctomorpha: Liposcelididae, *Liposcelis* sp., *Embidiopsocus* sp.

Trogiomorpha, Trogidae, *Cerobasis* sp.

Sternorrhyncha

Aleyrodoidea: Aleyrodidae, *Aleyrodes* sp.

Aphidoidea: Drepanosiphidae, *Drepanosiphum plantanoides* (Schrank, 1801); Aphididae, *Microsiphum* sp.

Coccoidea: Pseudococcidae, *Pseudococcus* sp; Coccidae, *Coccus* sp.

Psylloidea: Psyllidae, *Cacopsylla* sp.

Thysanoptera

Terebrantia: Thripidae, *Frankliniella* sp.

Zoraptera

Zorotypidae: *Zorotypus weidneri* New, 1978.

Outgroups

Hymenoptera, Xyelidae: *Xyela* sp.
Neuroptera, Nevrorthidae, *Nevrorthus* sp.
Orthoptera, Caelifera, Acridiidae: *Locusta migratoria* (L., 1758).
Orthoptera, Ensifera, Tettigoniidae: *Tettigonia viridissima* (L., 1758).
Odonata, Gomphidae, *Ictinus angulosus selys*, 1854.
Plecoptera, Nemouridae, *Nemoura cinerea*, Latreille 1796.

Results

Tarsal morphology

Auchenorrhyncha (Figs 1 and 2). Fulgoromorpha, Cixiidae, and Delphacidae (Fig. 1c,j,k). The tarsi of all examined species are 3-segmented and an arolium (ar, Fig. 1c) is present. In delphacids two strong bristles (Weirauch, 2005: guard setae) are inserted dorsally on the distal part of the tarsus.

In *Cixius* sp. the distal tarsomere of the fore- and midlegs is slightly longer than the others, whereas in the hindleg the proximal tarsomere is almost three times as long as the two distal ones. The apices of the two basal segments each bear two thick bristles. The unguitractor plate of *Cixius* sp. (Fig. 1j) and *Javasella* sp. (Fig. 1k) is characterized by a washboard-like surface. It is subdivided into small sclerotized platelets in three columns and 16 rows in *Cixius* sp., whereas they are arranged in two columns and seven rows in *Javasella* sp.

Cicadomorpha

Membracoidea, Membracidae, and Cicadellidae (Fig. 1b,d,g,h). The tarsi are 3-segmented. The proximal segment is the smallest. The dorsal side of the pretarsus appears scaly. A bilobed arolium (bar, Fig. 1b,d) is present. The arolium is largely fused with the claws. Only the tip of the claw is free. The surface of the unguitractor plate appears scaly (Fig. 1g,h). In *Centrotus* the entire dorsolateral region of the pretarsus is strongly sclerotized (dls, Fig. 2c). Medially adjacent to this sclerotized area is a large plate-like sclerite (ms, Fig. 2c). A sensillum (sen, Fig. 1d) protrudes at the inner distal corner of this structure. No sclerites are present on the ventral side of the pretarsus.

In *Cicadella* the dorsolateral regions of the arolium are also heavily sclerotized. The medial sclerites (ms, Fig. 2d) are present, but triangular and much smaller than those of *Centrotus*. These sclerites also bear a sensillum (sen, Fig. 1b). The sensilla in *Cicadella viridis* appear more delicate than those of *Centrotus cornutus*.

Cicadoidea, Cicadidae (Fig. 1a). The tarsi are 3-segmented. No specific attachment structures are present in *Cicadetta montana*. A single sensillum is present between the claws. Whether this sensillum is homologous with the "dorsal arolium" of enicocephalids is unclear. Three thick bristles are arranged in a row on the ventral base of the claws. Ventrally the tarsomeres are densely covered with short setae. Additionally, a long and thin sensillum is present on the ventral side of the first and third tarsomere.

Cercopoidea, Cercopidae (Figs 1e,f and 2a,b). The tarsi are 3-segmented. An arolium is present. It is medially distinctly incised. A protrusion with a vestiture of microtrichia (mt, Fig. 1e,f) is present on the distolateral region of the arolium, directly below the claws. A sclerotized bar bearing 3–4 thick bristles (sb, Figs 1i and 2a) is present ventrolaterally of the arolium on each side of the pretarsus. Dorsally a long sclerite (dls, Fig. 2b) is directly adjacent to the claws. These dorsolateral sclerites enclose a V-shaped medial sclerite (ms, Fig. 2b). Three setae are inserted on each side of the distal part of the arms of the "V". There are no differences between the pretarsi of *Philaenus* and *Cercopis*.

Sternorrhyncha (Fig. 3)

Coccoidea, Pseudococcidae, and Coccidae. In *Pseudococcus* and *Coccus* the tarsus is composed of one segment (Fig. 3a). Only one claw is present. On each side of the base of the claw a capitate fleshy structure (termed "claw digitule" by Cockerell, 1893 and Kondo, 2006) is present. Dorsally two long, slender setae with capitate ends (= tarsal digitules, tadi) are present. The structure of the claw digitules strongly suggests that they are homologous with pulvilli.

Aphidoidea, Drepanosiphidae, and Aphididae. The tarsi are 2-segmented (Fig. 3b,c). An eversible, cushion-like pad (tip, Fig. 3b) is present between the tarsus and tibia in *Microsiphum* sp. and *Aphis sambuci*. Additionally, setiform parempodia are located on the pretarsus.The pad between tibia and tarsus is absent in *Drepanosiphum* sp., but fleshy pulvilli (referred to as "empodial pads" in Kennedy, 1986) are present (pu, Fig. 3c). There is no difference in the attachment structures between winged and wingless morphs. Some species of *Neophyllaphis* bear two eversible adhesive

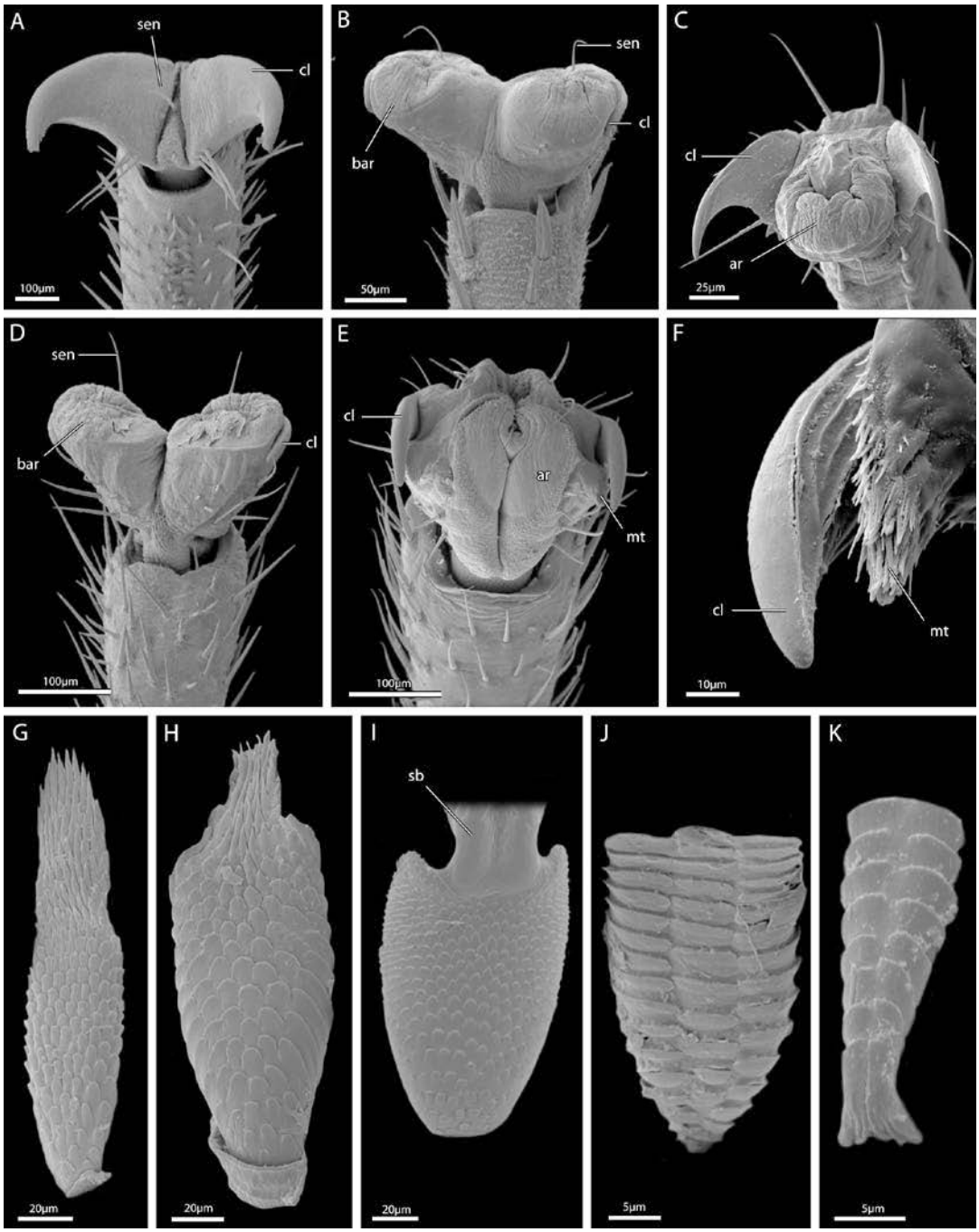

Fig. 1. Tarsi of Auchenorrhnycha. (a) *Cicadetta montana* (Cicadidae), ventral view. (b) *Cicadella viridis* (Cicadellidae), ventral view. (c) *Cixius* sp. (Cixiidae), frontal view. (d) *Centrotus cornutus* (Membracidae), ventral view. (e) *Cercopis vulnerata* (Cercopidae), ventral view. (f) *Cercopis vulnerata*, detail view of the protrusion covered in microtrichia. (g–k) unguitractor plates. (g) *Cicadella viridis*. (h) *Centrotus cornutus*. (i) *Cercopis vulnerata*. (j) *Cixius* sp. (k) *Javasella* sp. ar, arolium; bar, bilobed arolium; cl, claw; mt, microtrichia; sb, sclerotized bar; sen, sensillum.

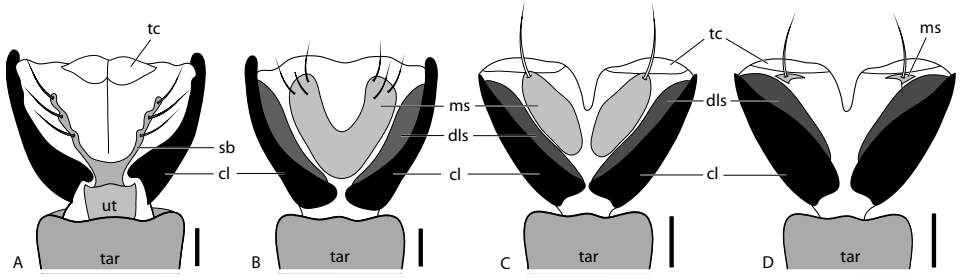

Fig. 2. Sclerites of pretarsi of Auchenorrhyncha. (a) *Cercopis vulnerata*, ventral view. (b) *Cercopis vulnerata*, dorsal view. (c) *Centrotus cornutus*, dorsal view. (d) *Cicadella viridis*, dorsal view. cl, claw; dls, dorsolateral sclerite; ms, medial sclerite; sb, sclerotized bar; tar, tarsus; tc, area of thickened cuticle; ut, unguitractor.

vesicles on the posterior abdominal sternites (Carver and White, 1971).

Psylloidea, Psyllidae. The tarsi of *Cacopsylla* are 2-segmented (Fig. 3d). A bilobed arolium (bar) is present. Three strong guard setae (gs) are present on the dorsal side of the tarsus. The distal part of the arolium is smooth, whereas the proximal part shows a rippled surface structure. Ventrally two setiform parempodia (par) arise from the unguitractor plate.

Aleyrodoidea, Aleyrodidae. The tarsi of the examined species are 2-segemented and covered with small wax platelets like the rest of the body (Fig. 3e). Dorsally one long guard seta is present. The two claws are largely covered with microtrichia from their bases to the middle region. They are thin and their tip remains glabrous. Between them a spine-like empodium (em) approximately as long as the claws is present. The base of this structure is also covered with microtrichia. The tip is flattened and glabrous with lamellae.

Most authors follow Quaintance and Baker (1913) in referring to this medial structure as "paronychium." However, Deshpande (1933) suggested to "treat the paronychium as an empodium rather than as a pulvillus".

Heteropterodea (Fig. 4)

Coleorrhyncha, Peloridiidae. The tarsi of *Hackeriella* are 2-segmented (Fig. 4c). An arolium is present between the claws.

Enicocephalomorpha. The tarsi are 2-segmented in *Systelloderes* (Fig. 4a,f). The distal tarsomere is about four times longer than the proximal one, and densely covered with long setae on all sides. There are no specific attachment structures. Two setiform parempodia (par, Fig. 4a) arise from the distal part of the unguitractor plate (ut). An alveolus is not recognizable. The unguitractor plate bears rectangular scales on its lateral side. The ventral distal rim of the distal tarsomere bears a row of microtrichia (= ventral brush after Weirauch, 2005). Dorsally between the claws, a so called "dorsal arolium" (ds, Fig. 4f) is present. This name is misleading as the structure is clearly not an arolium, but a sensillum (= dorsomedian sensillum after Weirauch, 2005). The foreleg in enicocephalids is distinctly modified for capturing prey. The tarsus comprises only one segment, and the distal part of the tibia bears an armature consisting of spiniform setae.

Dipsocoromorpha, Dipsocoridae, and Schizopteridae. The tarsi are 2-segmented in the species examined (Fig. 4b). The distal tarsomere is about three times longer than the proximal one. No specific pretarsal attachment structures are present in *Ceratocombus australiensis*. The lateral part of the unguitractor plate bears rectangular ridges. A ventral brush and setiform parempodia are missing, but two minute protuberances are present at the distal part of the unguitractor plate where the parempodia normally arise. These structures probably represent strongly reduced parempodia. It is very unlikely that this is an artifact as the same condition is found on all legs and a line of fracture is never recognizable. In the males of *Corixidea* there is an arolium present on the midlegs. There are no parempodia present on the midlegs, only on the hind- and forelegs. According to Stys (1983), metacoxal adhesive pads are present in Dipsocoromorphs. However, they were absent in all species examined.

Pentatomomorpha, Pentatomidae. The tarsi are 3-segmented in pentatomids (Fig. 4d,e). The ventral distal rim of the distal tarsomere bears a row of microtrichia, referred to as ventral brush (Weirauch, 2005). A small seta is present at the lateral end of the row. Two long setiform parempodia (par, Fig. 4d) arise from an alveolus on the distal part of the unguitractor plate. The ventral and lateral surfaces of the unguitractor plate bear distinct ridges. Large pulvilli (pu, Fig. 4d) are present. Their dorsal side is lamellate (Fig. 4e) and the ventral side more or less concave (variable among species).

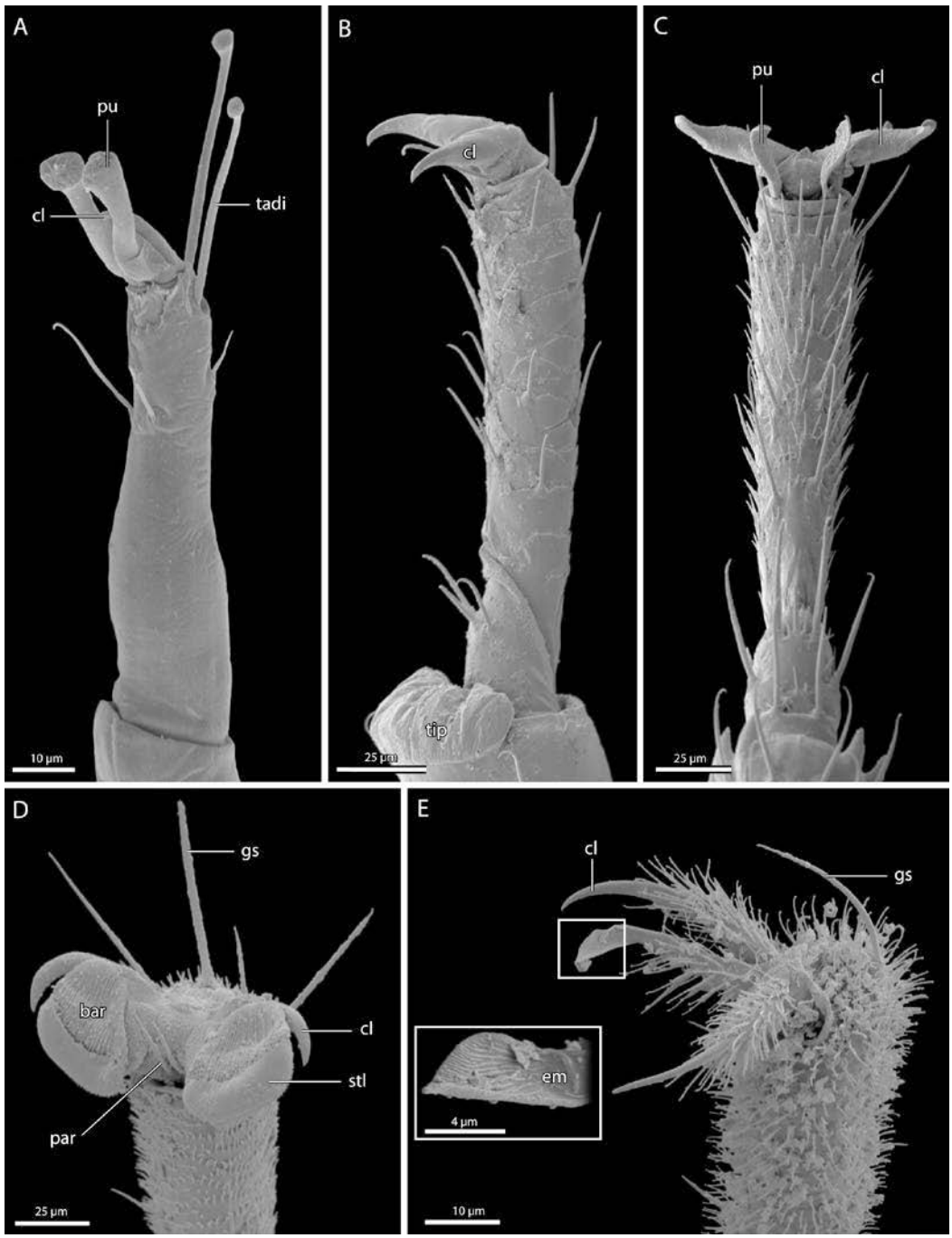

Fig. 3. Tarsi of Sternorrhyncha. (a) *Pseudococcus* sp. (Pseudococcidae), lateral view. (b) *Aphis sambuci* (Aphididae), lateral view. (c) *Drepanosiphum* sp. (Drepanosiphidae), ventral view. (d) *Cacopsylla* sp. (Psyllidae), frontal view. (e) *Aleyrodes* sp. (Aleyrodidae), lateral view. bar, bilobed arolium; cl, claw; em, empodium; gs, guard seta; par, parempodia; pu, pulvillus; stl, sticky lip of the arolium; tadi, tarsal digitules; tar, tarsus; ti, tibia; tip, tibial pad.

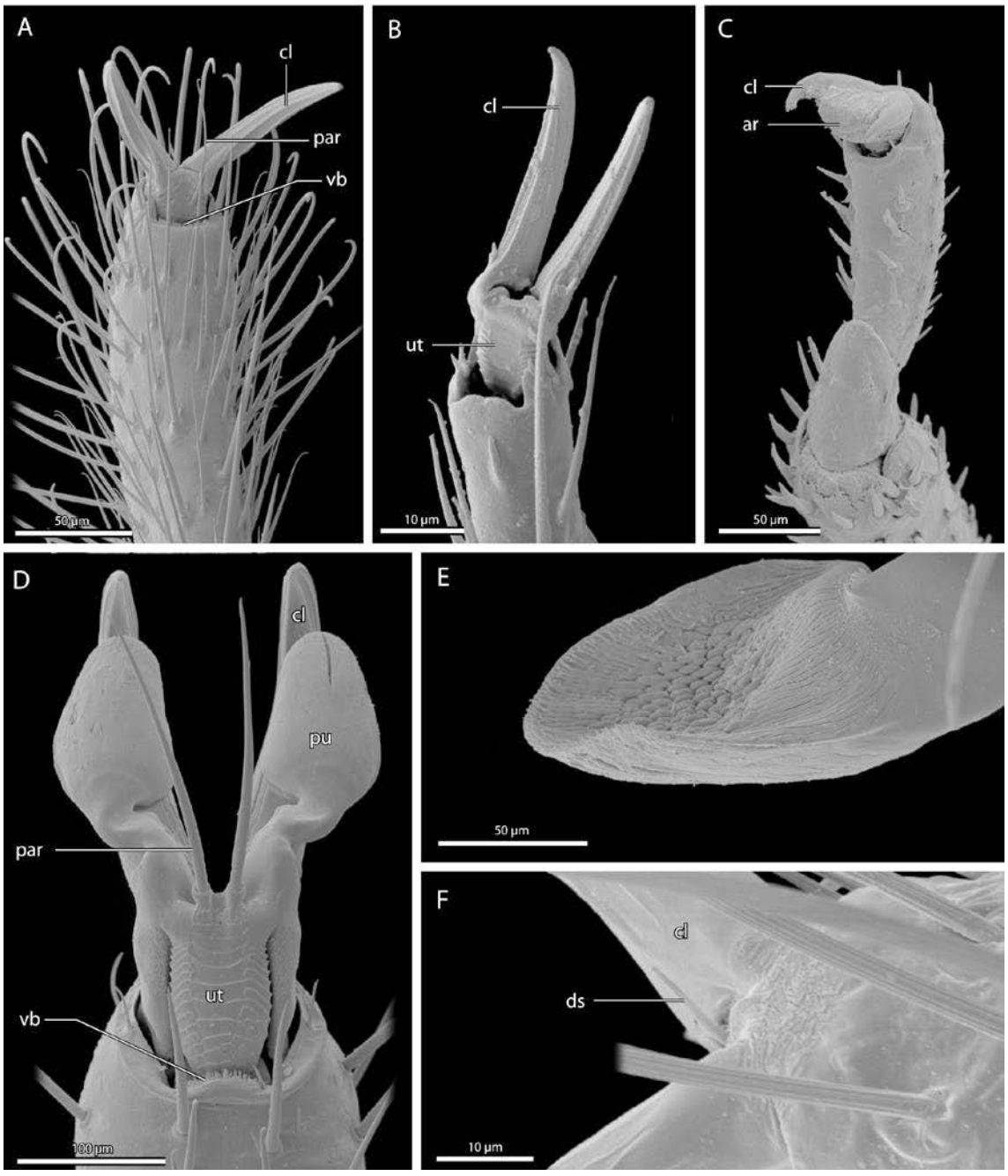

Fig. 4. Tarsi of Heteropterodea. (a) *Systelloderes* sp. (Enicocephalidae), ventral view. (b) *Ceratocombus australiensis* (Dipsocoridae), ventrolateral view. (c) *Hackeriella veitchi* (Peloridiidae), ventrolateral view. (d) *Graphosoma lineatum* (Pentatomidae), ventral view. (e) *Graphosoma lineatum* (Pentatomidae), dorsal surface of the pulvillus. (f) *Systelloderes* sp. (Enicocephalidae), dorsal view of the pretarsus. ar, arolium; cl, claw; ds, dorsal sensillum; par, parempodia; pu, pulvillus; ut, unguitractor; vb, ventral brush.

Psocodea (Fig. 5)

Troctomorpha, Liposcelididae. The tarsi are 3-segmented in *Liposcelis* (Fig. 5a). Adhesive structures are absent. The claws are serrate. The femur is distinctly enlarged. All tarsomeres, as well as the lateral and dorsal sides of the claws, are densely covered with microtrichia.

Psocomorpha, Caeciliidae. The tarsi are 2-segmented in *Caecilius* (Fig. 5b). Paired, flap-like pulvilli are present. Two guard setae are inserted on the dorsal side of the distal tarsal segment.

Trogiomorpha, Trogidae (Fig. 5c). Tarsi are 2-segmented in *Cerobasis*. Paired fleshy pulvilli are present. Two smooth claws are present (in other species claw teeth may be present, Yoshizawa, 2005). Three long, strong guard setae are located on the dorsal side of the distal tarsal segment. Directly proximad the pulvilli additional adhesive hairs (adh, Fig. 5c) are present [called "Basalhaare" in Weidner (1972, p. 50)]. They arise from the claws, not from the unguitractor plate.

Amblycera, Menoponidae. The tarsi are 2-segmented in *Trinoton* (Fig. 5d,g). The proximal tarsomere is smaller than the distal one and both bear smooth, slightly concave euplantulae (eu, Fig. 5d). The proximal surface of the euplantulae is covered with tubercles (tu, Fig. 5g). Two sensilla with a flag-like appearance (sen, Fig. 5d) are located at the ventral base of the proximal tarsomere. Two claws are present.

Ischnocera, Trichodectidae, and Philopteridae. The tarsus is 1-segmented and only one claw is present in *Trichodectes melis* (Trichodectidae) (Fig. 5e). Three to five stout, cone-like hyaline structures (hyc) are present on the apex of the tibia. In ischnoceran species parasitizing birds (*Columbicola* sp., Philopteridae), two claws are present.

Anoplura, Pediculidae. The tarsus is 1-segmented and only one large claw is present (Fig. 5f). A thumb-like process (thp) is present at the distal part of the tibia. It is opposed to the claw and combined, both structures enclose the hairshaft of the host. At the ventral side of the tarsus a round, pad-like euplantula (eu) is present. A claw-shaped apophysis (apo) arises from it. At the base of the claw a fingerlike process, possibly with sensory function (Soler-Cruz and Martin-Mateo, 2009), is present.

Rhynchophthirina, Haematomyzidae. The tarsus is 1-segmented (Fig. 5h). There is one main claw, and directly above is a smaller accessory claw ("Nebenkralle" after Weber, 1969). The accessory claw (acl) is present only on the mid- and hindlegs. No specific attachment structures are present.

Thysanoptera, Terebrantia, Aeolothripidae. The tarsi are 2-segmented in *Frankliniella* (Fig. 6a,b). An eversible, balloon-shaped pretarsal structure is a modified arolium (ar, Fig. 6b). In retracted condition it is encased by two spoon-shaped valves (va, Fig. 6a). Those valves are reduced claws (see Heming, 1971a, 1971b). Distally these valves are covered with tooth-like microtrichia (mt).

Character coding of tarsal structures (for character coding of other structures, see Appendix 2)

Coding as (0) or (1) does not imply a priori polarity assessment. We do not follow the convention of coding presumably plesiomorphic characters as (0). We consistently coded the adhesive structures of the middle leg.

99. Parempodia on unguitractor plate: (0) absent; (1) elongate and setiform, inserted in an alveolus.

100. Number of tarsal segments: (0) one; (1) two; (2) three; (3) more than three.

101. Arolium: (0) absent; (1) present; (2) eversible; (3) bilobed.

102. Sticky terminal lip of arolium: (0) absent; (1) present.

103. Pulvilli: (0) absent; (1) present.

104. Euplantulae: (0) absent, (1) present.

105. Number of claws: (0) one; (1) two; (2) reduced; (3) main claw plus accessory claw.

106. Claw teeth: (0) absent; (1) present.

107. Protuberance with microtrichia on distolateral side of the pretarsus: (0) absent; (1) present.

108. Sensorial setae on mesal side of arolium: (0) absent; (1) present.

109. Adhesive claw setae: (0) absent; (1) present.

110. Eversible structure between tibia and tarsus: (0) absent; (1) present.

111. Tibial thumb-like process: (0) absent; (1) present.

112. Empodial paronychium: (0) absent; (1) present.

113. Tarsal apophysis on the ventral side of the tarsus: (0) absent; (1) present.

114. Two dorsal capitate setae: (0) absent; (1) present.

115. Flag-like sensilla on the 1st tarsal segment: (0) absent; (1) present.

116. Fingerlike process below claw: (0) absent; (1) present.

117. Ventral brush: (0) absent; (1) present.

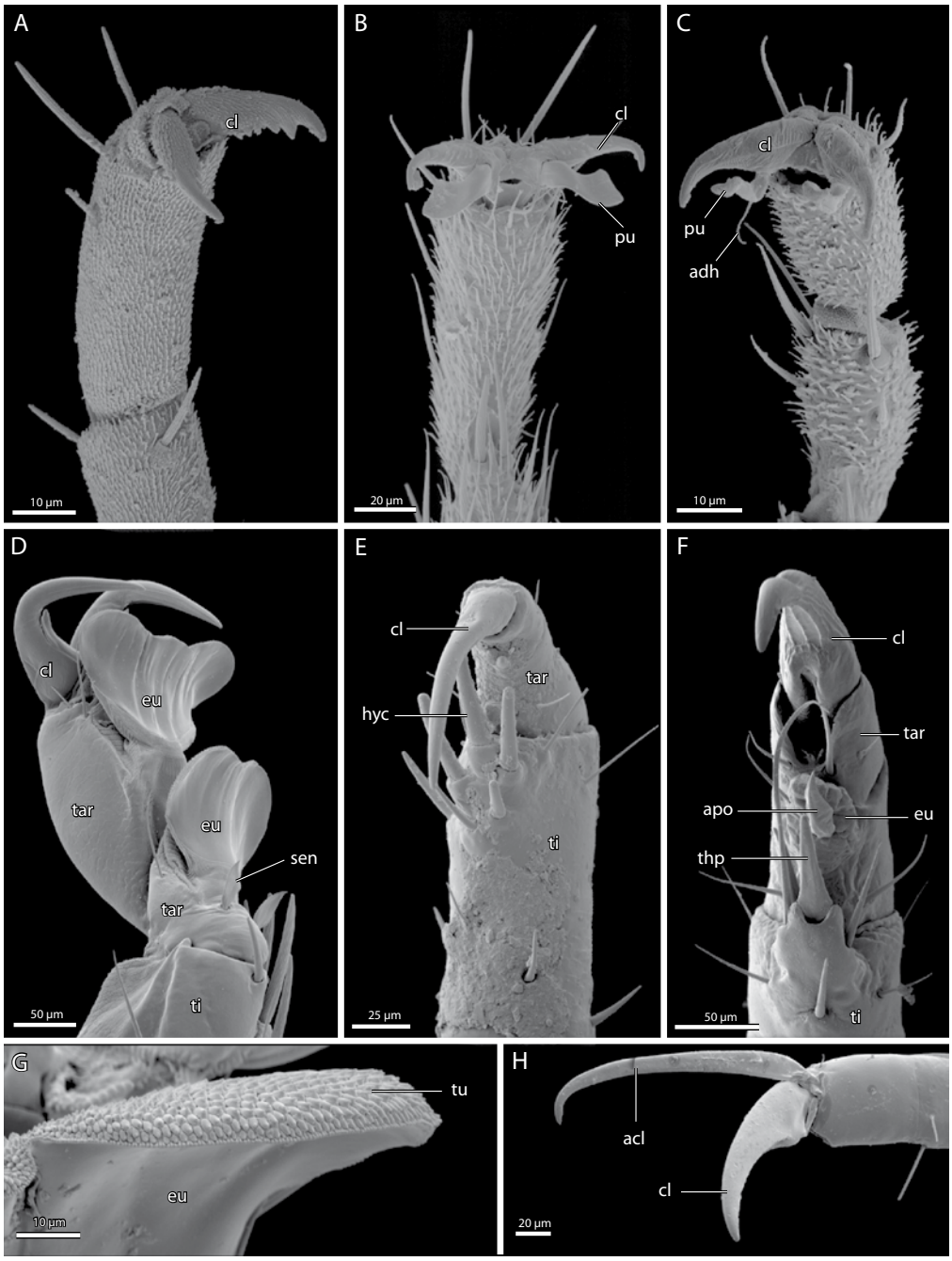

Fig. 5. Tarsi of Psocodea. (a) *Embiopsocus* sp. (Troctomorpha, Liposcelididae), lateral view. (b) *Caecilius flavidus* (Psocomorpha, Caeciliidae), ventral view. (c) *Cerobasis* sp. (Trogiomorpha, Trogidae), ventral view. (d) *Trinoton anserinum* (Amblycera, Menoponidae), lateral view. (e) *Trichodectes melis* (Ischnocera, Trichodectidae), ventral view. (f) *Pediculus humanus* (Anoplura, Pediculidae), ventral view. (g) *Trinoton anserinum* (Amblycera, Menoponidae), detail of the dorsal surface of the euplantulae. (h) *Haematomyzus elephantis* (Rhynchophthirina, Haematomyzidae), lateral view. acl, accessory claw; adh, adhesive hair; apo, apophysis of the euplantulum; cl, claw; eu, euplantulae; hyc, hyaline cones; pu, pulvillus; tar, tarsus; thp, thornlike process; ti, tibia; tu, Tubercle.

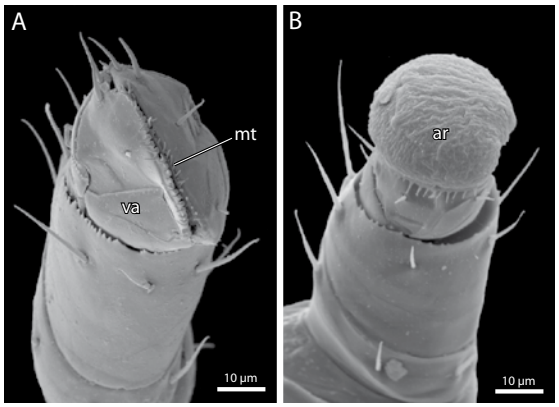

Fig. 6. Tarsi of Thysanoptera. (a) *Frankliniella* sp. Valves closed around the resting arolium. (b) *Frankliniella* sp. Arolium fully extended. ar, arolium; mt, microtrichia; va, valves.

Cladistic analysis

The analysis of 118 characters yielded three most parsimonious trees (195 steps, Ci: 68, Ri: 84). The strict consensus tree is shown in Fig. 7. Adhesive pad characters are mapped on the tree in Fig. 8. Apomorphies of ingroup taxa (character optimization: unambiguous) are listed in the following. Homoplasious changes are in italics. Further information about the characters can be found in Appendix 1 and 2.

Acercaria [Bremer support (BS): 4]

Lacinia stylet-like (7/1), single complex formed by abdominal ganglia (12/2), anterior region of 2nd axillary sclerite inflated (26/1), *fusion of gonangulum with tergum IX (44/1)*, number of Malpighian tubules reduced (93/2), *two tarsal segments (100/1)*.

Psocodea (BS: 5)

Rupture-facilitating modification at base of antennal flagellum (0/1), cardo and stipes fused (4/2), cibarial water-vapour uptake apparatus (10/1), two axonemes in spermatozoa (43/2), *arolium absent (101/1)*.

Liposcelididae + True lice (BS: 4)

Head and body dorsoventrally flattened (86/1), *hind-femora enlarged (87/1)*, meso- and metanotum fused (88/1), *compound eyes reduced (89/1)*.

True lice ("Phthiraptera",BS: 2)

Number of antennal flagellomeres reduced (58/1), ovipositor simplified (80/1).

True lice excl. Amblycera (BS: 2)

Maxillary palps absent (41/1), broad basal apodeme (63/1) and partly fused ventral plates (64/1) of the male genitalia, *only one tarsal segment (100/0)*, *single claw (105/0)*.

Rhynchophthirina + Anoplura (BS: 5)

Mandible stylet-like (3/1), lacinia absent (5/0), *cibarial water-uptake apparatus absent (10/0)*, *articulations between the mesomere*, *anterodorsal extension of ventral plate and posterior end of basal plate of the genitalia absent(59/0)*, *mesomere of the aedeagus pointed posteriorly (66/1)*, posteromedian part of basal plate of male genitalia sclerotized (67/1), *proboscis present (77/2)*, pronotum and procoxae fused (83/1), anterior tentorial pits absent (84/1), *hind femora not enlarged (87/0)*.

"Condylognatha" (Hemiptera + Thysanoptera; BS: 1)

Mandibles stylet-like (3/1), distal median plate of forewing positioned next to second axillary sclerite and articulating along convex hinge (33/1), *maxillary palps absent or reduced to less than four segments (41/1)*, *proboscis present (77/2)*, dorsal shift of anterior tentorial pits (84/2), labrum narrowed (94/1).

Hemiptera (Auchenorrhyncha, Heteropterodea, and Sternorrhyncha; BS: 3)

Cardo absent (4/1), labial rostrum present (8/1), anterior axillary folding-line forked around distal end of second axillary sclerite, proximal branch running through distal portion of 2Ax (27/1), tubular labium comprising three segments (55/1).

Auchenorrhyncha (BS: 4)

Proximal median plate of the forewing membraneous (31/1), *Evan's organ present (73/1)*, complex tymbal acoustic system present (91/1), *antennal flagellum aristate (92/1)*, *three tarsal segments present (100/2)*.

Fulgoromorpha (BS: 4)

Tegulae enlarged with broad extension encircling entire margin (20/1), pretentorium unites internal extremities of mandibular lever and corpotentorium (45/1), sensory plate organs of pedicel present (72/1), *arolium with sticky terminal lip (102/1)*.

Cicadomorpha (Bremer support: 1)

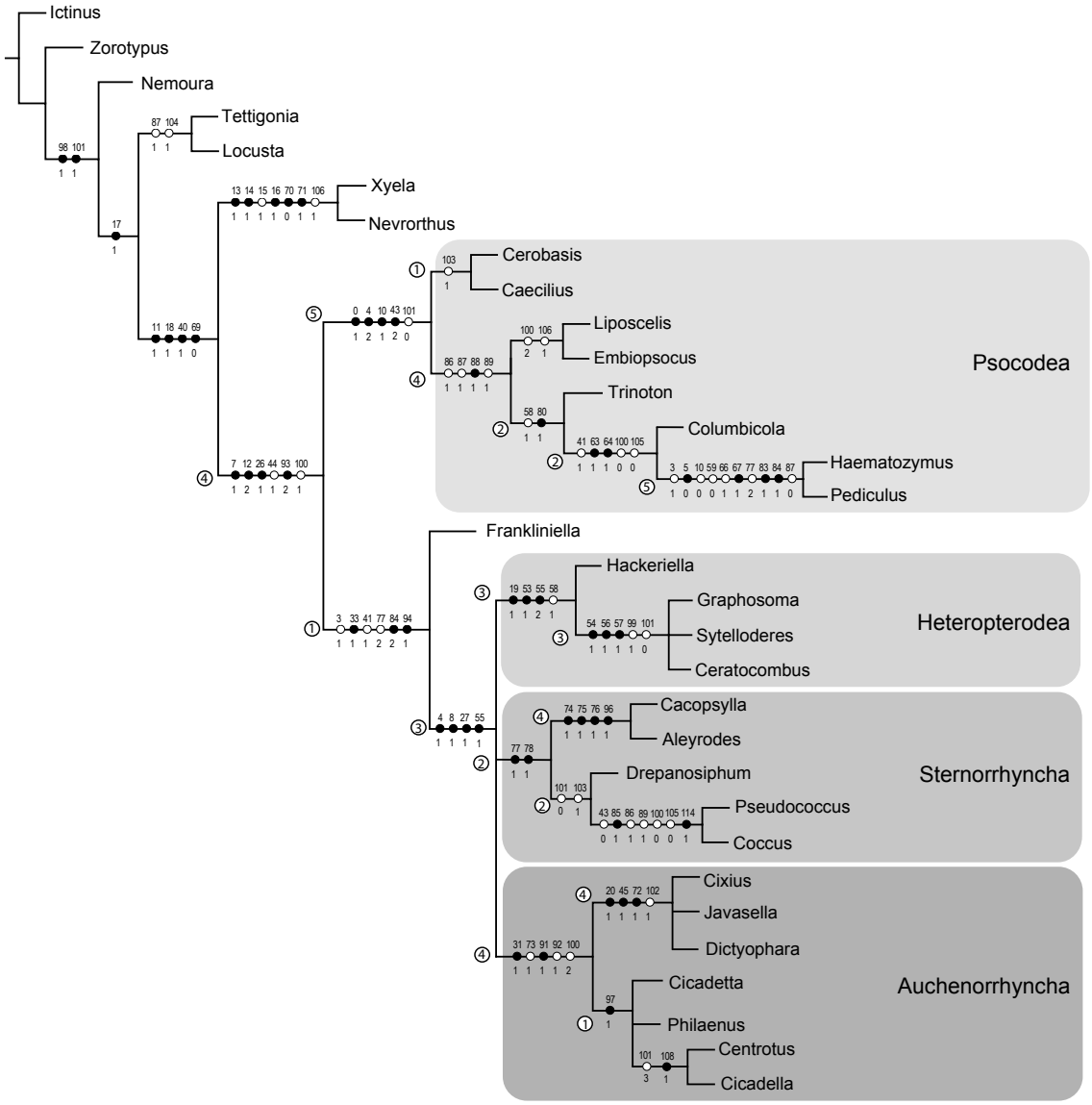

Fig. 7. Strict consensus cladogram of 118 characters (195 steps, Ci: 68, Ri: 84, unambiguous only). Black circles indicate non-homoplasious apomorphies, white circles homoplasious ones. Encircled numbers indicate Bremer Support values, which were calculated using TNT.

Gut with filter chamber containing Malpighian tubules (97/1).Heteropterodea (Coleorrhyncha + Heteroptera; BS: 3).

Tegulae of forewing absent (19/1), cephalic trichobothria (53/1), tubular labium comprising four segments (55/2), *number of flagellomeres of the antenna reduced (58/1)*.

Heteroptera (BS: 3)

Metathoracic scent gland system (54/1), labial proboscis inserted anteriorly on head (56/1), dorsal abdominal glands present in immature stages(57/1), *parempodia on pretarsus present (99/1)*, *arolium absent (101/0)*.

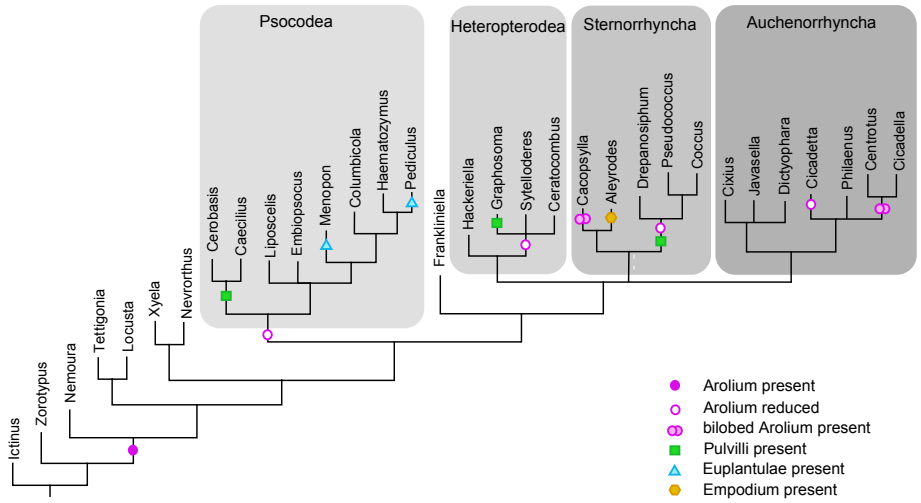

Fig. 8. Strict consensus cladogram of 118 characters, adhesive pad characters mapped on cladogram.

Sternorrhyncha (BS: 2)

Proboscis shifted posteriorly between procoxal bases (77/1), posterior parts of head capsule membraneous (78/1).

Psyllidae + Aleyrodidae (BS: 4)

Ductus ejaculatorius modified as sperm pump (74/1), abdomen narrowed by reduction of segments I and II (75/1), hind coxae broad and closely adjacent (76/1), eggs pedunculate (96/1).

Aphidoidea + Coccoidea (BS: 2)

Arolium absent (101/0), *pulvilli present (103/1)*.

Discussion

Phylogenetic aspects

The phylogenetic relationships of Acercaria were discussed informally by Hennig (1969), in several review studies by Kristensen, 1981; Kristensen et al., 1991); and also briefly by Trautwein et al. (2012). The placement and phylogeny of the entire lineage were addressed in several studies based on molecular data sets (e.g. Wheeler et al., 2001; Kjer, 2004, 2006) and also the phylogenetic relationships of the subgroups (e.g. Wheeler et al., 1993; Yoshizawa and Johnson, 2010; Cryan and Urban, 2012). Aside from studies covering the entire Hexapoda (Beutel and Gorb, 2001, 2006), the first numerical analysis of morphological data including all orders of Acercaria was conducted by Yoshizawa and Saigusa (2001), based on characters of the base of the forewing. In the present study we attempt to compile and analyse a more extensive morphological data set, including characters of all body parts. In the following, the results of the analyses are compared with those obtained in earlier studies based on different data, and especially with respect to the evolution of attachment structures, the primary focus of this study.

Acercaria

The monophyly of Acercaria is well supported by our data set (Bremer support: 4). The Psocodea, Thysanoptera, and Hemiptera share a set of synapomorphic features of different body regions, such as a stylet-like lacinia, arguably a predisposition for specialized sucking–piercing feeding habits, an inflated anterior region of the 2nd axillary sclerite, an extremely compacted abdominal ganglionic chain, and a reduced number of Malpighian tubules (shared with Holometabola excl. Hymenoptera; Beutel et al., 2011). The analysis based on our taxon sampling yielded a 2-segmented tarsus as an additional acercarian autapomorphy. This interpretation appears questionable as 3-segmented tarsi occur in several lineages [e.g. Psocoptera (partim), Heteroptera (majority of groups)]. Parallel loss of the 3rd tarsomere appears more plausible than a secondary acquisition in different groups. The slow optimization yielded strongly reduced labial palps and the absence of the abdominal sternite 1 as additional apomorphies of the Acercaria.

Our results do not support a placement of Zoraptera as the sister-group of Acercaria (e.g. Hennig, 1969; Beutel and Weide, 2005). The precise position of this

small and enigmatic order is not settled yet. However, there is an increasing consensus that they should be placed among the lower neopteran lineages (e.g. Kukalová-Peck and Peck, 1993; Wheeler et al., 2001; Yoshizawa and Johnson, 2005; Yoshizawa, 2007; Ishiwata et al., 2011; Yoshizawa, 2011; see also Trautwein et al., 2012).

The placement of Acercaria as sister-group of Holometabola is widely accepted even though poorly supported by morphological data (e.g. loss of larval ocelli; e.g. Beutel and Gorb, 2001, 2006). A clade including Acercaria and Holometabola (Eumetabola) is also tentatively supported by our data (with a very limited holomatabolan taxon sampling) and by molecular studies (e.g. Kjer, 2004; Ishiwata et al., 2011). However, this requires further confirmation. An arrangement with paraphyletic Acercaria and Psocodea as sister-group of Holometabola, as shown in Ishiwata et al. (2011), appears very unlikely considering the morphological evidence.

Psocodea

A clade Psocodea is well supported by unique morphological features (Figs 7 and 8; Rudolph and Knülle, 1982; Seeger, 1975) as well as molecular data (Murrell and Barker, 2005; Cryan and Urban, 2012; Ishiwata et al., 2011). A highly unusual apomorphic groundplan feature identified by Seeger (1975) is the cibarial water-uptake apparatus. An additional apomorphy probably present in the groundplan of Psocodea is the mortar-and-pestle apparatus of the cibarium (e.g. Tröster, 1990). It is still retained in the groundplan of Phthiraptera but is reduced in the majority of its subgroups (e.g. Tröster, 1990).

The relationships within Psocodea are still not fully clarified. However, a sister-group relationship between Liposcelididae and the true lice seems to be well supported by morphological characters such as enlarged hindfemora and fused pterothoracic nota (Figs 7 and 8; see also Lyal, 1985) and also by analyses of molecular data (Yoshizawa and Johnson, 2003; 12S, 16SrDNA). This renders the "Psocoptera" paraphyletic. The Phthiraptera (true lice) were weakly supported as a monophyletic unit (Bremer support: 2) in our analyses. Potential apomorphies are the reduced number of antennal flagellomeres, a condition also occuring in Heteroptera and Coleorrhyncha, and the simplified ovipositor (well developed in the psocodean groundplan). An entire series of apomorphies was suggested by Königsmann (1960), such as the absence of ocelli in all stages, the posteriorly tilted protocerebrum (see also Tröster, 1990), and a fusion of the metathoracic ganglion with the abdominal complex. Moreover, the complete reduction of the flight organs (absent or distinctly reduced in Liposcelididae), a dorsoventrally flattened body (also in Liposcelididae), and ectoparasitic habits were considered as obvious candidates for phthirapteran autapomorphies (see e.g. Grimaldi and Engel, 2005). Despite this seemingly strong morphological evidence, the monophyly of the true lice was questioned with respect to Amblycera in recent studies based on molecular data. Analyses of 18SrDNA (Johnson et al., 2004; Murrell and Barker, 2005) yielded a clade Amblycera + Liposcelididae on one hand, and the remaining true lice as its sister-group. This hypothesis suggests that parasitism in this lineage has evolved twice independently and also a series of features characterizing the four ectoparasitic groups. In an analysis using five different genes (nuclear 18S rDNA, Histone 3, wingless, mitochondrial 16S rDNA, and COI; Yoshizawa and Johnson, 2010) those result were supported. However, the results of the study by Murrell and Barker (2005) also include the unlikely paraphyly of Hemiptera and an unorthodox placement of Coleorrhyncha as sister-group of Auchenorrhyncha. Considering the morphological data and the specialized ectoparasitism on mammals and birds, we consider a clade Phthiraptera as more likely, but further confirmation by more extensive molecular data is required.

The branching pattern obtained within the true lice corresponds with the phylogenetic hypotheses suggested in earlier morphological studies (Lyal, 1985; Tröster, 1990) and a study based on 18SrRNA (Barker et al., 2003). The basal placement of Amblycera (Königsmann, 1960; Lyal, 1985; Tröster, 1990; see also Grimaldi and Engel, 2005) was confirmed in our analyses. Unambiguous synapomorphies of Ischnocera, Rhynchophthirina, and Anoplura (Fig. 7) are modifications of the male genital apparatus, i.e. the presence of broad basal apodemes and partly fused ventral plates. The sister-group relationship between Rhynchophthirina and Anoplura (e.g. Lyal, 1985; Tröster, 1990) is supported by an entire series of synapomorphies (Fig. 7) such as stylet-like mandibles (like in Hemiptera), loss of the lacinia, secondary absence of the cibarial water-uptake apparatus, the fusion of the procoxae with the pronotum, and the absence of anterior tentorial pits. Species of Anoplura and Rhynchophthirina feed exclusively on liquid, like the hemipterans. The food substrate is exclusively blood of birds or mammals in the former group, whereas this is a rare exception in the case of the Hemiptera (e.g. Cimicidae and some Reduviidae). The underlying structural modifications differ fundamentally in both lineages (Weber, 1929; Tröster, 1990).

Condylognatha

The placement of Thysanoptera is a matter of longstanding controversy (e.g. Kristensen et al., 1991). The characters we analysed support a clade Condylognatha

(Fig. 7; Bremer support 1), i.e. a sister-group relationship between Thysanoptera and Hemiptera. Potential synapomorphies include the stylet-like mandibles (right mandible vestigial in thrips), a specifically articulated distal median plate of the forewing, the reduction of the maxillary palps (absent in Hemiptera, fewer than four segments in thrips), and a dorsal shift of the anterior tentorial pits. The same result was obtained in several studies using different morphological character sets (e.g. Kristensen, 1981; Yoshizawa and Saigusa, 2001; Wheeler et al., 2001; : fig. 10; see also Hennig, 1969) and was also tentatively supported by molecular data analysed by Ishiwata et al. (2011). The alternative hypothesis, a clade Micracercaria (Thysanoptera + Psocodea), is suggested by the presence of an enlarged dorsal cibarial muscle with an unpaired median tendon (e.g. Willmann and Dathe, 2005). A sister-group relationship between Thysanoptera and Psocodea was also tentatively supported by analyses of 18S rDNA and 28S rDNA (Wheeler et al., 2001) and a study using seven gene regions (Cryan and Urban, 2012). As in the study of Ishiwata et al. (2011), the sampling of psocodeans and thrips was very limited in Cryan and Urban's (2012) study, which focused on hemipteran relationships. The results of Wheeler et al. (2001) have to be taken with caution. Neither the analyses of 18SrRNA nor those of 28SrRNA (Wheeler et al., 2001; : figs 13 and 14) supported a clade only containing the psocodean and thysanopteran terminals. Moreover, the analytical procedure (POY, simultaneous alignment and parsimony analyses) has been shown to be less reliable than other approaches (Kjer et al., 2007; Ogden and Rosenberg, 2007; Yoshizawa, 2010).

Hemiptera

There is no doubt about the monophyly of Hemiptera (Auchenorrhyncha, Heteropterodea, and Sternorrhyncha; e.g. Hennig, 1969; Kristensen, 1981; Kristensen et al., 1991). The most conspicuous autapomorphy is the characteristic labial rostrum, with reduced palps and endite lobes. This finding is clearly supported by our own data (Fig. 7) and also by molecular studies using different data sets and analytical approaches (Kjer, 2006; Ishiwata et al., 2011; Cryan and Urban, 2012). The paraphyly of Hemiptera and an unlikely sister-group relationship between Thysanoptera and Sternorrhyncha were suggested in a study based on SSUrDNA (Murrell and Barker, 2005). However, the focus was on the relationships within Psocodea, and the sampling of outgroups (in this case Thysanoptera and Hemiptera) was limited.

Our data turned out to be insufficient for resolving the interrelationships of the three hemipteran subgroups. Schuh (1979) suggested Sternorrhyncha as the sister-group of the remaining three lineages, thus rendering "Homoptera" paraphyletic. The same conclusion was reached by Popov (1981, palaeontological data), Zrzavy (1992, morphological and ecological data) and Cryan and Urban (2012, extensive molecular data). A taxon consisting of Heteropterodea (= Prosorrhyncha) and Auchenorrhnycha was referred to as Euhemiptera. This was also supported in several studies analysing different partial sequences of 18SrDNA (Campbell et al., 1995; Dohlen and Moran, 1995; Sorensen et al., 1995).

Auchenorrhyncha

A clade Auchenorrhyncha (Bremer support: 4) was well supported by our data (Figs 7 and 8). The presence of a complex tymbal acoustic system appears to be a convincing argument for this clade. Within the group, Fulgoromorpha were also clearly confirmed as a monophyletic unit (Bremer support: 4). The monophyly of Auchenorrhyncha was also supported by analyses of sequences of a broad array of genes (Urban and Cryan, 2007; 18S rDNA, 28S rDNA, Histone 3, Wingless) and in an even more extensive study using seven gene regions (Cryan and Urban, 2012; 18S rDNA, 28S rDNA, histone H3, histone 2A, wingless, cytochrome *c* oxidase I, NADH dehydrogenase subunit 4). However, it was challenged in other studies. A sister-group relationship between Cicadomorpha and Aphidoidea was suggested based on characters of the head capsule by Hamilton (1981), and a closer relationship between fulgorids and true bugs was proposed by von Dohlen and Moran (1995). The latter study was based only on 18SrRNA, and the taxon sampling was very limited, with only nine species of Auchenorrhyncha included. Similarities in the morphology and histology of the digestive tract of Fulgoromorpha and Heteroptera were pointed out by Goodchild (1966; for a summary see Forero, 2008), arguably a result of parallel evolution. A sister-group relationship between Cicadomorpha and Heteropterodea (Heteroptera + Coleorrhyncha) appears as a serious alternative to the monophyly of Auchenorrhyncha. This was suggested in an evaluation of combined paleontological, molecular, and morphological data (Bourgoin and Campbell, 2002) and also supported by a recent study based on transcriptomes (Letsch et al., 2012). Considering the conflicting hypotheses, the issue of the monophyly of Auchenorrhyncha should be considered an unsolved question.

Heteropterodea (= Prosorrhyncha, Coleorrhyncha + Heteroptera)

Coleorrhyncha are clearly placed as the sister-group to Heteroptera (Fig. 7; Bremer support: 3). The same

result was supported by Cryan and Urban (2012) and in other studies (Wheeler et al., 1993; Ouvrard et al., 2000; see also Schlee, 1969), and also by a detailed comparative study of head structures (Spangenberg et al., in press). Furthermore, the wing-coupling structure of moss bugs is very similar to that of Heteropterans (D'Urso, 1993). Considering the overwhelming evidence from different sources, previous placements of Coleorrhyncha as a subordinate group within Heteroptera (Breddin, 1897) or Auchenorrhyncha (China, 1962; Murrell and Barker, 2005) can be regarded as obsolete. Structural affinities of Coleorrhyncha and members of Auchenorrhyncha include features of the heart (occupying six abdominal segments and with six sets of alary muscles; Pendergrast, 1962) and the presence of an arolium. We assume that these similarities are either symplesiomorphic (e.g. arolium) or results of parallel evolution.

The monophyly of Heteroptera was clearly confirmed (Bremer support: 3), whereas the relationships within the group remained unresolved. This is mostly due to the very fragmentary knowledge of the morphology of supposedly basal groups such as Enicocephalomorpha and Dipsocoromorpha (Wheeler et al., 1993; Xie et al., 2008; summarized by Weirauch and Schuh, 2011). The basal branching events in Heteroptera are not yet fully clarified. A recent analysis of multiple genes yielded a basal position of Nepomorpha (Li et al., 2012). The position of Enicocephalomorpha and Dipsocoromorpha varied in the Li et al. (2012) study depending on the method applied (maximum likelihood versus maximum parsimony). The placement of Nepomorpha at the base of Heteroptera would be consistent with findings of Mahner (1993, p. 15ff), who considered the reduction of the tentorium as an autapomorphy of Heteroptera excl. Nepomorpha. The tentorium of enicocephalids is largely reduced (R. Spangenberg, pers. obs.), but the condition in dipsocoromorphans is not yet known. The fast optimization search yielded one additional apomorphy for the Heteroptera: the presence of a ventral brush on the ventral distal rim of the distal tarsomere. However, it is absent in the members of Dipsocoromorpha examined.

Sternorrhyncha

Sternorrhyncha were clearly confirmed as a clade (Fig. 7). Autapomorphies are the posterior shift of the proboscis between the procoxal bases and the membraneous posterior parts of the head capsule. A sister-group relationship between Psyllidae and Aleyrodidae (Psyllomorpha) was also well supported (Bremer support: 4), which is not surprising as most characters were taken from Schlee (1969), who proposed this hypothesis. Synapomorphies are the ductus ejaculatorius modified as a sperm pump, the constriction of the abdominal base, the broad and closely adjacent hind coxae, and the pedunculate eggs. The only potential synapomorphy of aphids and coccids is the loss of the arolium. However, the arolium is lost several times within Acercaria.

In several studies based on 18S rDNA (Campbell et al., 1995; Sorensen et al., 1995), Psyllidae were placed as the sister-group of the remaining Sternorrhyncha, and Aleyrodidae as the sister-group of a clade comprising of Aphidoidea and scale insects. The same relationships were inferred from DNA nucleotid sequence data from seven gene regions (Cryan and Urban, 2012). Whiteflies share at least some morphological features with aphids and scale insects, such as reduced wing venation, sedentary or sessile nymphs, and antennae reduced to six or fewer segments (e.g. Grimaldi and Engel, 2005). Apparently the interrelationships of the sternorrhynchan subgroups require further investigation.

Evolution of attachment structures

Despite a very broad spectrum of structural variations, there are only two basic designs of attachment pad on insect legs: hairy and smooth (Beutel and Gorb, 2001). Interestingly, hairy structures that occur in different lineages of Polyneoptera and Holometabola (Beutel and Gorb, 2001, 2006) are lacking on the tarsus and pretarsus of the acercarian subgroups. An interesting exception is the hairy fossula spongiosa in several subgroups of Cimicomorpha. This is the only case of a hairy pad in Acercaria and, apart from tibial elements of some specialized clasping devices of true lice, the only tibial attachment structure occurring in the entire Hexapoda. The exact function of the fossula spongiosa is still unclear. It is possibly a tool for capturing prey, or alternatively a device involved in locomotion (Weirauch, 2007). It was pointed out by Beutel and Gorb (2001) that the function of adhesive devices of insect legs is generally not restricted to attachment. Easy detachment is equally important to guarantee efficient locomotion on a specific substrate.

The location of different attachment devices within Acercaria varies considerably. They occur on the pretarsus as an unpaired pad-like arolium, as paired pulvilli, or as an unpaired sclerotized empodium; on the tarsus as smooth euplantulae; and (as noted above) even on the distal tibia as a fossula spongiosa. This and the incompletely resolved relationships within Hemiptera impede the reconstruction of the evolutionary pathways. However, it is noteworthy that pretarsal attachment structures are mainly present in the primarily phytophagous Hemipterans, whereas tarsal and tibial attachment devices occur mostly in the ectoparasitic lice (Phthiraptera). In these taxa they are essential

for anchoring on the hairs or feather shafts of the host.

Some groups lack attachment devices completely, for example the ground-dwelling Zoraptera (Beutel and Gorb, 2006). They are also absent in Enicocephalomorpha, female Dipsocoromorpha, Gerromorpha, Nepomorpha, Leptodomorpha, Rhynchophthirina, and Troctomorpha. With the exception of the ectoparasitic Rhynchophthirina and the semi-aquatic and aquatic Gerromorpha and Nepomorpha, all these taxa are ground-oriented and live in leaf litter, in microhabitats similar to those of the zorapterans. The elephant lice do not attach to hairs in the typical case, but stay directly on the skin surface or in skin creases (Fowler and Mikota, 2006; p. 458). Their main anchoring mechanism appears to be the proboscis, which is firmly embedded in the skin of the host (Weber, 1969).

Arolium

Arolia are widely spread within Acercaria and a groundplan feature of this lineage. They are generally present in the Auchenorrhyncha examined (with the exception of Cicadidae), in Coleorrhyncha, and in Thysanoptera. An arolium is also present in the males of the genus *Corixidea* and several other male members of the Schizopteridae (Emsley, 1969; p. 20). The females of Dipsocoromorpha lack specific adhesive structures. The distinctly bilobed arolium of membracids and leafhoppers (Membracidae and Cicadellidae; Figs 1b,d and 2c,d; and Psylloidea; Fig. 3d) has apparently evolved independently in these groups, as potential autapomorphies of these lineages. A unique and apparently autapomorphic feature is the eversible, balloon-like arolium of Thysanoptera. In its retracted position it is enclosed between two valves. This is not the case in fulgorids, where the arolium is also eversible to a certain degree (Frantsevich et al., 2008).

The arolium was considered as a potential autapomorphy of Neoptera by Beutel and Gorb (2001, 2006). However, whether the unpaired pretarsal attachment pads occurring in different insect lineages are homologous is questionable. The sclerotization of the arolium of sawflies (Gladun, 2008), for instance, is completely different from the pattern found in the arolia of members of Cicadomorpha. The sclerotized Y-shaped ridge on the ventral side of the arolium in cercopoids may represent strongly modified planta, but this would imply that the auxillae and the arcus are reduced. The V-shaped sclerite on the dorsal side of the pretarsus of cercopids could be a modified manubrium. However, this interpretation is also uncertain. Due to the uncertain assessment of homologies, it is presently not possible to decide whether the differences between arolia occurring in acercarian and holometabolan lineages are due to secondary modifications of substructures or to nonhomology of the unpaired pretarsal pads as a whole.

In contrast, there is little doubt that the unmodified arolium of cercopoids and the bilobed arolium in membracids are homologous. This is clearly indicated by specific conformities in the inner structure and sclerotization of these devices. In both cases, the cuticle is thickened and composed of branched chitinous rods. A modification characterizing the Membracoidea examined is the loss of the ventral Y-shaped sclerite. A characteristic feature of membracids is the division of the medial sclerite into two separate elements (ms, Fig. 2c). It is V-shaped but undivided in cercopoids. In cicadellids these sclerites are greatly reduced in size (ms, Fig. 2d), arguably an autapomorphy of the family.

The homology of the attachment device of whiteflies is still unclear. In this study we reluctantly label it as an empodium. Most authors, however, follow Quaintance and Baker (1913) in referring to this structure as "paronychium" (= arolium). An arolium is defined as a median hollow lobe of the pretarsus (Dashman, 1953; Beutel and Gorb, 2001). The structure in the Aleyrodidae examined is not hollow, but resembles a spine-like empodium with a flattened tip. Median empodia are usually covered with acanthae (Beutel and Gorb, 2001). This applies only to the base of the structure occurring in Aleyrodidae. This and the absence of empodia in related groups suggests that this is a de novo formation and autapomorphy, rather than a structure homologous to the empodia occurring in several holometabolan lineages (e.g. Diptera).

The results of our analysis suggest the secondary loss of the arolium in several lineages. This includes Heteroptera, Aphidoidea, Coccoidea, and Psocodea. In all these groups, arolia were apparently functionally replaced by other kinds of attachment device, in most cases by paired pulvilli. Considering the relationships indicated by our analysis (Fig. 8) and other studies, it appears that the loss of the arolium is an autapomorphy of each of the taxa listed above. However, as a complete reduction of this structure also occurs in many other insect lineages (Beutel and Gorb, 2001, 2006), this feature is of minor phylogenetic significance.

Pulvilli

Pulvilli have evolved at least twice independently within Acercaria—in Psocoptera and in the "higher" Heteroptera, respectively (Fig. 7). Interestingly, pretarsal or tarsal adhesive pads are absent in most of the presumably basal taxa of examined Heteroptera, the Enicocephalomorpha, the semi-aquatic and aquatic lineages Gerromorpha and Nepomorpha, and also in Saldidae (Leptopodomorpha), predaceous and ground-

dwelling bugs, and arguably the sister-group of the remaining secondarily terrestrial heteropteran lineages (Wheeler et al., 1993). An arolium is present in Coleorrhynchans and some male members of the Schizopteridae (Dipsocoromorpha), and this is certainly a groundplan condition in Heteropterodea (and Acercaria). The loss of this unpaired pretarsal pad is probably a groundplan feature and autapomorphy of Heteroptera. Interestingly, in the megadiverse heteropteran subgroups Pentatomomorpha (ca. 14 500 spp.) and Cimicomorpha (more than 20 000 spp., Weirauch and Schuh, 2011) a novel type of attachment device occurs: paired pretarsal pulvilli. The presence of these adhesive structures is arguably related to a close association between those bugs and plants. As noted above, pretarsal and tarsal adhesive devices are absent in saldids. There is, however, one noteworthy exception: a structure resembling an empodium, similar to that of whiteflies, occurs in the genus *Aepophilus*. Interestingly, this attachment device, considered as a "true" arolium by Cobben (1978, p. 114 ff), is present only in fifth-instar nymphs and is completely lacking in all other stages. However, a "dorsal arolium" occurs in the adults of *Aepophilus bonnairei* (Schuh and Polhemus, 1980) and other saldids. Therefore it seems likely that Cobben misinterpreted this structure. He also stated the presence of arolia in Gerromorpha. However, the structures he described rather resemble paired accessory claws, and they were absent in species of *Gerris* we examined.

Pulvilli are also present in aphids and coccoids, apparently a result of parallel evolution. The pulvilli of coccoids are usually fleshy and thick, and therefore similar to those of the pentatomids examined. In contrast, the pulvilli of aphids are flattened like those found in trogiomorphs and psocomorphs (Psocodea). The shape of pulvilli can be very variable. This is particularly obvious in the Miridae (Schuh, 1976). However, the characteristic thickened cuticle with chitinous rods found in arolia is absent in the pulvilli examined.

Euplantulae and tibial pads

Within Phthiraptera no pretarsal attachment structures occur. The complete reduction of the arolium is probably related to ectoparasitic habits and a potential autapomophy of true lice. Within the group, different tarsal and tibial devices have evolved. A specific type of euplantulae is present in amblycerans, especially in species specialized on birds as hosts. They occur on both tarsomeres in Menoponidae, but only on the proximal segment in Laemobothriidae. Interestingly, euplantulae of the proximal tarsomere are also present in species of the genus *Paraheterodoxus* (Boopidae) (Marshall, 2003), even though these amblycerans live not on birds, but in the fur of the rufous rat-kangaroo. This is an example of how attachment devices of the same type can adapt to different surfaces or structures.

In Ischnocera and Amblycera the mandibles appear to play a more important role as grasping devices than the specialized structures on the legs (Bush et al., 2006). In specimens preserved in ethanol we observed that the hair shaft was not in contact with the legs any more, but was still firmly anchored between the mouthparts. In anoplurans the thumb-like process on the distal part of the tibia and the opposing claw enclose the hair. It is plausible to assume that the grasping mechanism is enhanced by the tarsal euplantulae. The same function is conceivable for the thick hyaline cones on the apex of the tibia in the Ischnocera examined. These cones on the tibial apex are apparently typical for many ischnoceran species specialized on birds (Smith, 2001).

Conclusions

As pointed out by Beutel and Gorb (2001), attachment devices can provide phylogenetic information despite functional constraints. In our study, the impact on the branching pattern was limited to an improved resolution within Psocodea. However, additional apomorphies of adhesive devices strengthened the support for different lineages (Psocodea, Fulgoromorpha, Heteroptera). Linked with a remarkable versatility in habitat choice, a very wide spectrum of attachment devices has evolved in Acercaria, with the notable exception of hairy pretarsal adhesive structures. Several acercarian subgroups can be characterized by features of the distal leg elements related to attachment and efficient locomotion on different substrates. The phylogenetic hypothesis presented here was based on a relatively limited character system and taxon sampling. In the near future, an extensive molecular data set will probably provide a robust phylogenetic framework for Acercaria and other hexapod lineages (see www.1KITE.org). This will be an ideal basis for reconstructing the evolution of attachment structures and other character systems.

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Appendix 1

Character state matrix

(?) Refer to missing character states; (–) refer to inapplicable characters.

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Ictinus	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	?	?	?	?	?
Nemoura	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tettigonia	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	?	?	?	?	?
Locusta	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	?	?	?	?	?
Xyela	0	0	0	0	0	1	0	0	0	1	0	1	0	1	1	1	1	1	1	0	?	1	?	?	?
Nevrorthus	0	0	0	0	0	1	0	0	0	1	0	1	0	1	1	1	1	1	1	0	?	?	?	?	?
Zorotypus	0	0	0	0	0	1	0	0	0	1	0	?	1	0	0	0	0	0	0	0	0	0	?	?	?
Trogiomorpha	1	0	0	0	2	1	1	1	0	0	1	1	2	0	0	0	0	1	1	0	0	0	1	1	0
Liposcelis	1	?	0	0	2	1	1	1	0	0	1	1	2	0	0	0	0	1	1	0	0	0	1	1	0
Embidiopsocus	1	?	0	0	2	1	1	1	0	0	1	1	2	0	0	0	0	1	1	?	?	?	?	?	?
Caecilius	1	0	0	0	2	1	1	1	0	0	1	1	2	0	0	0	0	1	1	0	0	0	1	1	0
Trinoton	1	0	0	0	2	1	1	1	0	0	1	–	2	0	–	0	0	1	1	–	–	–	–	–	–
Trichodectes	1	0	0	0	2	1	1	1	0	0	1	–	2	0	–	0	0	1	1	–	–	–	–	–	–
Haematomyzus	1	?	0	1	2	0	–	–	0	0	0	–	2	0	–	0	0	1	1	–	–	–	–	–	–
Pediculus	1	?	0	1	2	0	–	–	0	0	0	–	2	0	–	0	0	1	1	–	–	–	–	–	–
Frankliniella	0	1	1	1	0	1	0	1	0	1	0	1	2	0	0	0	0	1	1	0	0	1	1	2	1
Cacopsylla	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	0	0	0	1	2	0
Aleyrodes	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	?	?	?	?	?	?
Drepanosiphum	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	0	0	0	1	2	0
Pseudococcus	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	?	?	?	?	?	?
Coccus	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	?	?	?	?	?	?
Centrotus	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	0	0	0	1	2	0
Cicadella	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	0	0	0	1	2	0
Cicadetta	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	0	0	0	1	2	0
Cercopis	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	0	0	0	1	2	0
Cixius	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	0	1	0	1	2	0
Javasella	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	0	1	0	1	2	0
Dictyophora	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	0	1	0	1	2	0
Pentatomorpha	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	1	–	–	1	2	0
Systelloderes	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	1	–	–	1	2	0
Ceratocombus	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	1	–	–	?	?	?
Hackeriella	0	2	0	1	1	1	1	1	1	0	0	1	2	0	0	0	0	1	1	1	–	–	?	?	?

	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Ictinus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	1	0	1	1	0	0	0	0	?
Nemoura	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	1	1	0	0	0	0	0	?
Tettigonia	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	0	1	0	0	0	0	0	?
Locusta	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	0	1	0	0	0	0	0	?
Xyela	?	0	?	?	?	?	?	?	?	?	?	?	?	?	?	1	0	0	1	0	0	0	0	0	?
Nevrorthus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	0	0	1	0	?	?	0	0	?
Zorotypus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	3	0	0	1	0	0	0	1	0	?
Trogiomorpha	1	1	0	1	1	1	0	1	0	0	1	0	0	1	1	1	0	1	2	1	0	?	?	0	?
Liposcelis	1	1	0	1	1	1	0	1	0	0	1	0	0	1	1	1	0	1	2	1	0	0	0	0	?
Embidiopsocus	?	?	?	?	?	?	0	?	?	?	?	?	?	?	1	1	0	1	2	1	0	0	0	0	?
Caecilius	1	1	0	1	1	1	0	1	0	0	1	0	0	1	1	1	0	1	?	1	0	0	0	0	?
Trinoton	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	0	1	?	?	0	1	0	0	?
Trichodectes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	1	1	2	?	0	1	0	0	?
Haematomyzus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	1	1	?	?	0	0	0	0	?
Pediculus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	1	1	2	?	0	0	0	0	?
Frankliniella	0	1	0	1	1	1	0	?	1	1	1	1	1	1	0	3	1	0	3	1	0	0	0	?	?
Cacopsylla	1	1	1	1	1	1	0	1	1	0	1	0	0	1	0	2	1	1	1	1	0	0	0	?	?
Aleyrodes	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	2	1	1	0	1	?	?	0	?	?
Drepanosiphum	1	1	1	1	1	1	0	1	1	0	1	0	0	1	0	2	1	1	1	1	0	0	0	0	?
Pseudococcus	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	2	1	1	0	1	?	?	?	?	?
Coccus	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	2	1	1	0	1	?	?	?	?	?
Centrotus	1	1	1	1	1	1	1	1	1	0	1	0	0	?	0	2	1	1	1	1	0	0	0	0	?

(*Continued*)

	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Cicadella	1	1	1	1	1	1	1	1	1	0	1	0	0	?	0	2	1	1	1	1	0	0	0	0	?
Cicadetta	1	1	1	1	1	1	1	1	1	0	1	0	0	?	0	2	1	1	1	1	0	0	0	0	?
Cercopis	1	1	1	1	1	1	1	1	1	0	1	0	0	?	0	2	1	1	1	1	0	0	0	0	?
Cixius	1	1	1	1	1	1	1	1	1	0	1	0	0	?	0	2	1	1	1	1	1	0	0	0	?
Javasella	1	1	1	1	1	1	1	1	1	0	1	0	0	?	0	2	1	1	1	1	1	0	0	0	?
Dictyophora	1	1	1	1	1	1	1	1	1	0	1	0	0	?	0	2	1	1	1	1	1	0	0	0	?
Pentatomorpha	1	1	1	1	1	1	0	1	1	0	1	1	0	1	0	2	1	1	1	1	0	?	0	1	1
Systelloderes	1	1	1	1	1	1	0	1	1	0	1	0	0	1	0	2	1	1	?	?	0	0	0	0	0
Ceratocombus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	2	1	1	?	?	0	0	0	0	?
Hackeriella	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	2	1	1	?	1	0	0	0	0	?

	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74
Ictinus	?	?	0	0	0	0	–	0	1	?	?	?	?	?	?	?	?	?	?	1	1	0	0	–	0
Nemoura	?	?	0	0	0	0	–	0	0	?	?	?	?	?	?	?	?	?	?	1	1	0	0	–	0
Tettigonia	?	?	0	0	0	0	–	0	0	?	?	?	?	?	?	?	?	?	?	1	1	0	0	–	0
Locusta	?	?	0	0	0	0	–	0	0	?	?	?	?	?	?	?	?	?	?	1	1	0	0	–	0
Xyela	?	?	0	0	0	0	–	0	0	?	?	?	?	?	?	?	?	?	?	0	0	1	0	–	0
Nevrorthus	?	?	0	0	0	0	–	0	0	?	?	?	?	?	?	?	?	?	?	0	0	1	0	–	0
Zorotypus	?	?	0	0	0	0	–	0	0	?	?	?	?	?	?	?	?	?	?	1	1	0	0	–	0
Trogiomorpha	?	?	0	0	0	0	–	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	0	–	0
Liposcelis	?	?	0	0	0	0	–	0	0	1	–	0	0	0	0	0	0	0	0	0	1	0	0	–	0
Embidiopsocus	?	?	0	0	0	0	–	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	0	–	0
Caecilius	?	?	0	0	0	0	–	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	–	0
Trinoton	?	?	–	0	0	0	–	0	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	–	0
Trichodectes	?	?	–	0	0	0	–	0	1	1	0	0	0	1	1	1	0	0	0	0	1	0	0	–	0
Haematomyzus	?	?	–	0	0	?	?	0	1	0	0	0	0	1	1	0	1	1	0	0	1	0	0	0	0
Pediculus	?	?	–	0	0	?	?	0	1	0	0	0	0	1	1	0	1	1	1	0	1	0	0	?	0
Frankliniella	?	?	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	0	0	0
Cacopsylla	?	?	0	0	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	0	0	1
Aleyrodes	?	?	0	0	0	?	0	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	0	0	1
Drepanosiphum	?	1	0	0	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	0	0	0
Pseudococcus	?	?	0	0	0	?	0	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	0	0	0
Coccus	?	?	0	0	0	?	0	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	0	0	0
Centrotus	?	1	0	0	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	0	1	0
Cicadella	?	1	0	0	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	0	1	0
Cicadetta	?	1	0	0	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	0	1	0
Cercopis	?	1	0	0	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	0	1	0
Cixius	?	1	0	0	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	1	1	0
Javasella	?	1	0	0	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	1	1	0
Dictyophora	?	1	0	0	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	0	1	0	1	1	0
Pentatomorpha	1	1	1	1	1	2	1	1	1	?	?	?	?	?	?	?	?	?	?	0	1	0	0	0	0
Systelloderes	0	0	0	1	1	2	1	?	1	?	?	?	?	?	?	?	?	?	?	0	1	0	0	0	0
Ceratocombus	?	0	1	1	1	2	1	?	1	?	?	?	?	?	?	?	?	?	?	0	1	0	0	0	0
Hackeriella	1	1	0	1	0	2	0	0	1	?	?	?	?	?	?	?	?	?	?	?	?	0	0	1	0

	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99
Ictinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	?	0	–	0	0	0	0
Nemoura	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–	0	0	1	0
Tettigonia	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	–	0	0	1	0
Locusta	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	–	0	0	1	0
Xyela	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	–	?	0	0	0
Nevrorthus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–	0	0	1	0
Zorotypus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	–	0	0	0	0
Trogiomorpha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	?	1	0
Liposcelis	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	2	0	0	?	?	1	0
Embidiopsocus	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	2	0	0	?	?	?	0
Caecilius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	?	1	0
Trinoton	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	1	0	0	2	0	0	0	?	1	0
Trichodectes	0	0	0	0	1	1	1	1	0	0	0	1	1	1	1	1	0	0	2	0	0	0	0	1	0
Haematomyzus	0	0	2	0	1	1	1	1	1	1	0	1	0	1	1	1	0	0	2	0	0	0	0	1	0
Pediculus	0	0	2	0	0	0	0	0	1	1	0	1	1	1	1	1	0	0	2	0	0	0	0	1	0

(*Continued*)

	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99
Frankliniella	0	0	2	0	0	0	0	0	0	2	0	1	1	0	0	0	0	0	2	1	1	0	0	0	0
Cacopsylla	1	1	1	1	0	0	0	0	0	2	0	0	0	0	0	1	0	0	2	1	1	1	0	1	1
Aleyrodes	1	1	1	1	0	0	0	0	0	2	0	0	0	0	0	1	0	0	2	1	1	1	0	1	0
Drepanosiphum	0	0	1	1	0	0	0	0	0	2	0	0	0	0	0	1	0	0	2	1	1	?	0	1	0
Pseudococcus	0	0	1	1	0	0	0	0	0	?	1	1	0	0	1	1	0	0	2	1	1	0	0	1	0
Coccus	0	0	1	1	0	0	0	0	0	?	1	1	0	0	1	1	0	0	2	1	1	0	0	1	0
Centrotus	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	1	1	1	2	1	1	0	1	1	0
Cicadella	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	1	1	1	2	1	1	0	1	1	0
Cicadetta	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	1	1	1	2	1	1	0	1	1	0
Cercopis	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	1	1	1	2	1	1	0	1	1	0
Cixius	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	1	1	1	2	1	1	0	0	1	0
Javasella	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	1	1	1	2	1	1	0	0	1	0
Dictyophora	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	1	1	1	2	1	1	0	0	1	0
Pentatomorpha	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	2	1	1	0	0	1	1
Systelloderes	0	0	2	0	0	1	0	0	0	2	0	0	0	0	0	1	0	0	2	1	1	0	?	1	1
Ceratocombus	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	2	1	1	0	?	1	?
Hackeriella	0	0	2	0	0	0	0	0	0	2	0	1	0	0	0	1	0	0	2	1	1	0	?	1	0

	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117
Ictinus	3	0	–	0	0	1	1	–	–	0	0	0	0	0	0	0	0	0
Nemoura	2	1	0	0	0	1	0	–	–	0	0	0	0	0	0	0	0	0
Tettigonia	3	0	0	0	1	1	0	–	–	0	0	0	0	0	0	0	0	0
Locusta	3	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Xyela	3	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Nevrorthus	3	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Zorotypus	1	0	–	0	0	1	0	–	–	0	0	0	0	0	0	0	0	0
Trogiomorpha	1	0	–	1	0	1	0	–	–	1	0	0	0	0	0	0	0	0
Liposcelis	2	0	–	0	0	1	1	–	–	0	0	0	0	0	0	0	0	0
Embidiopsocus	2	0	–	0	0	1	1	–	–	0	0	0	0	0	0	0	0	0
Caecilius	1	0	–	1	0	1	0	–	–	0	0	0	0	0	0	0	0	0
Trinoton	1	0	–	0	1	1	0	–	–	0	0	0	0	0	0	1	0	0
Trichodectes	0	0	–	0	0	0	0	–	–	0	0	0	0	0	0	0	0	0
Haematomyzus	0	0	–	0	0	3	0	–	–	0	0	0	0	0	0	0	0	0
Pediculus	0	0	–	0	1	0	0	–	–	0	0	1	0	1	0	0	1	0
Frankliniella	1	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Cacopsylla	1	3	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Aleyrodes	1	1	0	0	0	1	0	–	–	0	0	0	1	0	0	0	0	0
Drepanosiphum	1	0	–	1	0	1	0	–	–	0	1	0	0	0	0	0	0	0
Pseudococcus	0	0	–	1	0	0	0	–	–	0	0	0	0	0	1	0	0	0
Coccus	0	0	–	1	0	0	0	–	–	0	0	0	0	0	1	0	0	0
Centrotus	2	3	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
Cicadella	2	3	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
Cicadetta	2	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Cercopis	2	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Cixius	2	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Javasella	2	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Dictyophora	2	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Pentatomorpha	2	0	–	1	0	1	0	–	–	0	0	0	0	0	0	0	0	1
Systelloderes	1	0	–	0	0	1	0	–	–	0	0	0	0	0	0	0	0	1
Ceratocombus	1	0	–	0	0	1	0	–	–	0	0	0	0	0	0	0	0	0
Hackeriella	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 2

Proposed phylogenetically informative characters

The list comprises 118 characters of the head, thorax, abdomen, and attachment structures. They were scored for 25 representatives of Acercaria. As outgroup taxa we chose one representative each of Odonata [*Ictinus angulosus Selys* (Mathur and Mathur, 1961)]; Plecoptera [*Nemouracinerea* (Moulins, 1968)]; Orthoptera [*Locusta migratoria* (Albrecht, 1953) and *Tettigona viridissima* (Henning, 1974)]; Hymenoptera [*Macroxyela ferruginea* (Vilhelmsen, 2000, 2001; Beutel and Vilhelmsen, 2007)]; Neuroptera [*Nevrorthus* sp. (Friedrich and Beutel, 2010)]; and Zoraptera [*Zorotypus hubbardi* (Beutel and Weide, 2005; Friedrich and Beutel, 2008)].

Some characters were taken from the data matrices of other authors:

Main source	Character numbers
Beutel and Gorb (2001)	0–15
Yoshizawa and Johnson (2006)	19–38
Beutel and Gorb (2006)	16–18
Yoshizawa and Johnson (2006)	46, 59–68
Wheeler et al. (1993)	47–55
Beutel et al. (2011)	69–71

0. Rupturing mechanism at the base of the antennal flagellum: (0) absent; (1) present.

A rupture-facilitating cuticle modification is present in Psocodea (Seeger, 1975; Beutel and Gorb, 2001). It is absent in all other examined taxa under consideration.

1. Exposure of mouthparts: (0) largely or completely exposed; (1) left mandible enclosed in a pouch formed by anteclypeal wall, labrum, stipes, and hypopharynx; (2) bases of mandibular and maxillary stylets articulate inside head with mandibular and maxillary plates.

The left mandible is enclosed in a pouch in Thysanoptera (Mickoleit, 1963). The bases of the mandibular and maxillary stylets articulate inside the head with mandibular and maxillary plates in Hemiptera (Carver et al., 1991). The situation is unknown for Rhynchophthirina, Anoplura, and Troctomorpha (coded as ?).

2. Right mandible: (0) present; (1) reduced.

The right mandible is reduced in Thysanoptera (e.g. Mickoleit, 1963).

3. Shape of mandibles: (0) not stylet-like; (1) stylet-like.

Stylet-like mandibles are generally present in Hemiptera (Hamilton, 1981) and have evolved independently within Phthiraptera [Anoplura and Rhynchophthirina (Weber, 1969)]. The left mandible is transformed into a stylet-like structure in Thysanoptera whereas the right mandible is reduced (char. 2). Biting mandibles are present in Psocoptera, *Trinoton* sp. (Amblycera), and *Trichodectes* sp. (Ischnocera).

4. Cardo: (0) present; (1) strongly reduced or absent; (2) fused with stipes.

The cardo is strongly reduced or absent in Hemiptera. This was considered as an autapomorphy of this group by Kristensen et al. (1991). It is completely fused with the stipes in Psocoptera and Phthiraptera and might therefore be an autapomorphy for the Psocodea (Yoshizawa and Saigusa, 2003).

5. Lacinia: (0) absent; (1) present.

The lacinia is missing in Anoplura and Rhynchophthirina (Weber, 1969; Tröster, 1990).

6. Insertion of lacinia: (0) on stipes; (1) detached from stipes.

The lacinia is detached from the stipes in Acercaria (e.g. Risler, 1951; Hamilton, 1981; Kristensen et al., 1991; Beutel and Gorb, 2001; Yoshizawa and Saigusa, 2003). This character is coded as (–) for taxa without lacinae. The lacinia inserts on the stipes in Thysanoptera (Crampton, 1923).

7. Lacinia: (0) not elongate and stylet-like; (1) elongate and stylet-like.

A stylet-like lacinia is present in Acercaria (Badonnel and Grassé, 1951; Carver et al., 1991) and was considered an apomorphy of this group by Kristensen et al. (1991). This character is coded as (–) for taxa without lacinae.

8. Labial rostrum: (0) absent; (1) present.

A labial rostrum is present in Hemiptera (Hamilton, 1981) and was considered an apomorphy of this group by Kristensen et al. (1991).

9. Labial palps: (0) absent or strongly reduced; (1) comprising at least 2 segments.

Labial palps are absent or strongly reduced in Psocoptera (Yoshizawa, 2005), Odonata (Mathur and Mathur, 1961) and Hemiptera (Hamilton, 1981). They comprise two to five segments in Thysanoptera (Priesner, 1968) and four in Xyelidae [Hymenoptera (Beutel and Vilhelmsen, 2007)]. The labial palps of all other examined species bear three segments.

10. Cibarial water-vapour uptake apparatus: (0) absent; (1) present.

The cibarial water-vapour uptake apparatus (Rudolph and Knülle, 1982) was considered an apomorphy of Psocodea (Kristensen et al., 1991). However, it is absent in Rhynchophthirina and Anoplura (Lyal, 1985).

11. Jugal "bar": (0) absent; (1) present.

The presence of a sclerotized jugal bar was considered as a synapomorphy of Acercaria and Holometabola (Hamilton, 1971; Kristensen et al., 1991). The character was coded as (–) for taxa without wings.

12. Abdominal ganglia: (0) more than two separate ganglia; (1) two separate ganglia; (2) one single ganglionic mass.

Two separate abdominal ganglionic complexes are found in Zoraptera (Hennig, 1969). A single ganglionic mass is a possible autapomorphy of Acercaria (e.g. Pendergrast, 1962; Lyal, 1985; Carver et al., 1991; Kristensen et al., 1991).

13. Eyes of immature stages: (0) persist; (1) disintegrate or pulled back proximally into cerebrum.

In Holometabola the compound eyes and ocelli of adults are formed de novo during metamorphosis (e.g. Friedrich and Benzer, 2000). They persist in all examined hemimetabolous species.

14. External wing buds: (0) present; (1) absent.

External wing buds are absent in Holometabola (e.g. Kristensen, 1999; Beutel and Gorb, 2001), except for shallow convexities in secondary larvae of Strepsiptera (Beutel and Pohl, 2006). The character is coded as (–) for wingless taxa.

15. Pupal stage: (0) absent; (2) present.

A pupa generally occurs in Holometabola (e.g. Hymenoptera; (Hinton, 1971) and a similar stage occurs in Thysanoptera (Hennig, 1973). The pupa in Nevrorthidae is aquatic, which is probably an autapomorphy of this group (Aspöck et al., 2012). All other examined species do not have a pupal stage.

16. Appearance of compound eyes: (0) before ultimate immature stage; (1) in ultimate immature stage.

The compound eyes appear before the penultimate life stage in non-holometabolan insects and Strepsiptera (Beutel and Gorb, 2006).

17. Ocelli of immature stages: (0) present; (1) absent.

Ocelli are generally absent in nymphs or larvae of Acercaria and Holometabola. They are also missing in nymphs of some groups of Orthoptera. They are present in Zoraptera, Plecoptera and Odonata (e.g. Beutel and Gorb, 2006).

18. Cerci of immature stages: (0) present; (1) absent.

Cerci are absent in immature stages of Acercaria and Holometabola (Beutel and Gorb, 2006; Yoshizawa and Johnson, 2006), with the possible exception of Strepsiptera (Beutel et al., 2011).

19. Tegulae of the forewing: (0) present; (1) absent.

Tegulae are absent in Heteroptera (Yoshizawa and Saigusa, 2001) and Coleorrhyncha. The character was coded as (–) for wingless groups. The situation is unknown for Coccoidea, Aleyrodidae, and *Embiopsocus* sp. (coded ?).

20. Size and shape of Tegulae: (0) small; (1) enlarged, with broad extension encircling the entire margin.

The tegulae are distinctly enlarged with a broad extension encircling the entire margin in Fulgoromorpha (Yoshizawa and Saigusa, 2001). They are small in Plecoptera, Zoraptera, Trogiomorpha, Psocomorpha, Thysanoptera, Psylloidea, Aphidoidea, and Cicadomorpha. The character was coded as (–) for wingless taxa and groups without tegulae. The situation is unclear for Coccoidea, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera (coded ?).

21. Attachment of tegulae to body wall: (0) narrow; (1) broad.

The tegulae are broadly attached to the body wall in Thysanoptera (Yoshizawa and Saigusa, 2001) and Xyelidae (Hymenoptera, Hörnschemeyer, 2002). The character was coded as (–) in wingless insects and in those where the tegulae are absent. The situation is unclear for Coccoidea, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, and Neuroptera.

22. HP and BSc: (0) separated from each other; (1) connected with each other.

The humeral plate (HP) is connected with the basisubcostale (BSc) in Psocoptera, Thysanoptera, Aphidoidea, Psylloidea, Auchenorrhyncha, and Heteroptera (Yoshizawa and Saigusa, 2001). HP and BSc are separated from each other in Plecoptera. The character was coded as (–) in wingless insects. The situation is unknown for the remaining outgroups, Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, and *Embiopsocus* sp.

23. BSc: (0) distant from 2Ax; (1) closely adjacent with the anteroproximal corner of 2Ax; (2) fused with anteroproximal part of 2Ax.

The basisubcostale (BSc) is in close proximity to the anteroproximal corner of the second axillary sclerite (2Ax) in Psocoptera. The BSc is fused with the second axillary sclerite (Yoshizawa and Saigusa, 2001) in Thysanoptera, Psylloidea, Aphidoidea, Auchenorrhyncha, and Heteroptera. It is distant form the second axillary sclerite in Plecoptera. The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Dipsocoromorpha, Neuroptera, and Hymenoptera.

24. BR and HP + BSc: (0) fused with each other; (1) separated from each other.

The basiradiale (BR) and humerale plate (HP) + basisubcostale (BSc) are separated from each other in Thysanoptera (Yoshizawa and Saigusa, 2001). They are fused in all other taxa under consideration. The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

25. BR and 2Ax: (0) separated from each other; (1) fused.

The basiradiale (BR) and the second axillary sclerite (2Ax) are separated from each other in Thysanoptera and Plecoptera (Yoshizawa and Saigusa, 2001). They are fused in Psocoptera, Psylloidea, Aphidoidea, Auchenorrhyncha and Heteroptera. The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

26. 2Ax: (0) nearly flat; (1) anterior region inflated.

The anterior region of the second axillary sclerite (2Ax) is inflated in Acercaria (Yoshizawa and Saigusa, 2001). It is flat in Plecoptera and Xyelidae (Hymenoptera; Hörnschemeyer, 2002). The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, and Neuroptera.

27. Anterior axillary folding-line: (0) not forked; (1) forked around distal end of 2Ax, proximal branch running through the distal portion of 2Ax.

The anterior axillary folding-line is forked around the distal end of the second axillary sclerite (2Ax) and its proximal branch is running through the distal portion of the second axillary sclerite in Hemiptera (Yoshizawa and Saigusa, 2001). It is not forked in Plecoptera, Thysanoptera, and Psocoptera. The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

28. Position of PMP: (0) distad 2Ax; (1) posterodistad 2Ax.

The proximal median plate (PMP) is located posterodistad the second axillary sclerite (2Ax) in Acercaria (Yoshizawa and Saigusa, 2001). It is distad 2Ax in Plecoptera. The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

29. PMP: (0) nearly flat; (1) deeply concave.

The proximal median plate (PMP) is deeply concave in Acercaria, whereas it is nearly flat in Plecoptera (Yoshizawa and Saigusa, 2001). The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

30. PMP: (0) almost evenly sclerotized; (1) distal margin sclerotized more strongly than its other regions.

The distal margin of the proximal median plate (PMP) is more strongly sclerotized than its other regions in Psocoptera, Thysanoptera, Sternorrhyncha, and Heteroptera. It is evenly sclerotized in Plecoptera. The character was coded as (?) for Auchenorrhyncha by Yoshizawa and Saigusa (2001) since their PMP is reduced. The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

31. PMP: (0) well sclerotized; (1) reduced, often completely membranous.

The proximal median plate (PMP) is membranous in Auchenorrhyncha whereas it is strongly sclerotized in the other acercarian lineages and in Plecoptera (Yoshizawa and Saigusa, 2001). The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

32. DMP: (0) not divided; (1) divided into 2 sclerites.

The distal median plate (DMP) is divided into 2 sclerites in Acercaria (Yoshizawa and Saigusa, 2001). The character was coded as (?) for Thysanoptera as their DMP is reduced. The DMP is not divided in Plecoptera. The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

33. DMP: (0) distant from 2Ax; (1) placed next to 2Ax, articulating along a convex hinge.

The distal median plate (DMP) is placed next to the second axillary sclerite (2Ax), articulating along a convex hinge in Hemiptera and Thysanoptera. It is distinctly separated from the second axillary sclerite in Psocoptera and Plecoptera (Yoshizawa and Saigusa, 2001). The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

34. DMP: (0) large; (1) reduced in size.

The distal median plate (DMP) is reduced in size in Thysanoptera (Yoshizawa and Saigusa, 2001). The character was coded as (–) in wingless insects. It is large in Plecoptera and the remaining acercarian lineages. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

35. Distal arm of 3Ax and DMP: (0) articulating with each other; (1) not articulating with each other.

The distal arm of the third axillary sclerite (3Ax) does not articulate with the distal median plate (DMP) in Acercaria (Yoshizawa and Saigusa, 2001). They articulate with each other in Plecoptera. The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

36. Anterior arm of 3Ax: (0) present; (1) absent.

The anterior arm of the third axillary sclerite (3Ax) is absent in Thysanoptera and Pentatomomorpha (Yoshizawa and Saigusa, 2001). The character was coded as (–) in wingless insects. It is present in Plecoptera, Psocoptera, Sternorrhyncha, Auchenorrhyncha, Enicocephalomorpha, and Dipsocoromorpha. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

37. 3Ax and BA: (0) separate from posterior margin of forewing base; (1) attached to posterior margin of forewing base.

The third axillary sclerite (3Ax) and the basanale (BA) are situated on the posterior margin of the forewing base in Thysanoptera (Yoshizawa and Saigusa, 2001). The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

38. BA and PMP: (0) separate from each other; (1) fused with each other.

The basanale (BA) and the proximal median plate (PMP) are fused with each other in Psocoptera, Thysanoptera, Psylloidea, Aphidoidea, and Heteroptera. They are separated in Plecoptera. The character was coded as (?) in Auchenorrhyncha due to the reduction of the PMP in this taxon (Yoshizawa and Saigusa, 2001). The character was coded as (–) in wingless insects. The situation is unknown for Coccoidea, Coleorrhyncha, Dipsocoromorpha, Aleyrodidae, *Embiopsocus* sp., Odonata, Orthoptera, Neuroptera, and Hymenoptera.

39. Lateral hypopharyngeal arm (0) present; (1) absent.

The lateral hypopharyngeal arm (loral arm after Matsuda, 1965) is absent in Psocodea (Yoshizawa, 2005) and Zoraptera (Beutel and Weide, 2005). It is present in Thysanoptera, Auchenorrhyncha, Aphidoidea, Psylloidea, Pentatomomorpha, Enicocephalomorpha, Dipsocoromorpha, and Coleorrhyncha (Hamilton, 1981). The situation is unknown for Aleyrodidae, and Coccoidea.

40. Ovarioles: (0) not polytrophic; (1) polytrophic; (2) telotrophic; (3) panoistic.

Panoistic ovarioles are present in Zoraptera and Thysanoptera. Ovarioles of the telotrophic type are present in Hemiptera. Polytrophic ovarioles occur in Psocodea and some groups of Holometabola (Štys and Bilinski, 1990; Kristensen et al., 1991).

41. Maxillary palps: (0) present, with four segments or more; (1) absent or reduced number of segments.

Maxillary palps are absent in Odonata, Hemiptera (Spooner, 1938) and Phthiraptera, with the exception of Amblycera, which have 4-segmented maxillary palps (Marshall, 2003). The number of segments is reduced to three in Thysanoptera. The maxillary palps of all other taxa under consideration comprise four or more segments.

42. Abdominal sternite 1: (0) present; (1) absent.

The abdominal sternite 1 is absent in Acercaria (Kristensen, 1981) with the exception of Thysanoptera. It is also absent in Plecoptera (Illies, 1965).

43. Number of axonemes in spermatozoans: (0) zero; (1) one; (2) two; (3) three.

A biaxonemal flagellum is present in Ischnocera, Anoplura, Troctomorpha, and Trogiomorpha (Dallai and Afzelius, 1991). The flagellum has one axoneme in Zoraptera, Auchenorrhyncha (Kubo-Irie et al., 2003; Dallai et al., 2011; Zhang and Dai, 2012), Pentatomomorpha (Araújo et al., 2011), aphids and psyllids (Bào et al., 1997) and in all outgroups (e.g. Szöllösi, 1975; Quicke et al., 1992; Afzelius and Dallai, 1994). The spermatozoans of Aleyrodidae, and Coccoidea are aflagellate (Bào et al., 1997). The situation is unknown for Coleorrhyncha, Enicocephalomorpha, Amblycera, Rhynchophthirina, Psocomorpha, and Dipsocoromorpha (coded as ?). The flagellum of Thysanoptera has three amalgamated axonemes (e.g. Paccagnini et al., 2006, 2009).

44. Gonangulum: (0) not fused with tergum IX; (1) fused with tergum IX.

The gonangulum is fused with tergum IX in Acercaria and Odonata (Yoshizawa, 2005; Klass et al., 2012). The situation is unknown for Enicocephalomorpha, Dipsocoromorpha, and Phthiraptera.

45. Pretentorium: (0) absent or if present not connecting internal extremities of mandibular lever and corpotentorium; (1) unites internal extremities of mandibular lever and corpotentorium.

The pretentorium connects the internal extremities of the mandibular lever and corpotentorium in adult Fulgoromorpha (Bourgoin, 1986). This is not the case in the other taxa under consideration (see (Albrecht, 1953; Beutel and Vilhelmsen, 2007; Hamilton, 1981; Moritz, 2008). The situation is unknown for Neuroptera, Aleyrodidae, and Coccoidea.

46. Lacinial gland: (0) absent; (1) present.

A lacinial gland is present in Amblycera, Ischnocera, and some members of the Trogiomorpha (coded ? for *Cerobasis* sp.) (Yoshizawa and Johnson, 2006). It is absent in the remaining Psocoptera, Rhynchophthirina (Lyal, 1985), Odonata (Mathur and Mathur, 1961), Orthoptera (Albrecht, 1953), Xyelidae (Hymenoptera) (Beutel and Vilhelmsen, 2007), Zoraptera (Beutel and Weide, 2005), Thysanoptera (Mickoleit, 1963) and Auchenorrhyncha (Butt, 1943), Coleorrhyncha, Heteroptera, and Dipsocoromopha. The situation is unknown for Coccoidea, Aleyrodidae, and Neuroptera.

47. Male genitalia: (0) symmetrical, or if asymmetrical, asymmetry not involving pregenital segments; (1) asymmetrical, this asymmetry often involved pregenital segments.

Asymmetrical genitalia can be found in *Stemmocrypta* sp. (Dipsocoromorpha) (Stys, 1983) and in some Zoraptera (F. Hünefeld, pers. comm). The situation is unknown for Coccoidea and Trogiomorpha. The genitals of all described Ceratcombinae are symmetrical (Stys, 1982).

48. Accessory salivary glands generally: (0) not tubular; (1) of the tubular type.

The accessory salivary glands are generally of the tubular type in Pentatomomorpha (Baptist, 1941; Southwood, 1955; Wheeler et al., 1993). The situation is unknown in Thysanoptera, Psylloidea, Aleyrodidae, and Coccoidea.

49. Number of eye trichobothria of first instars: (0) one or absent; (1) two.

Enicocephalomorpha have less than two eye trichobothria in the first-instar larvae, whereas Pentatomomorpha have two trichobothria (Cobben, 1978; Schuh, 1979; Wheeler et al., 1993). The situation is unknown for all other taxa under consideration.

50. Number of ommatidia in first-instar larvae: (0) 4-5; (1) more than five.

More than five ommatidia are present in the first-instar larva of Coleorrhyncha and Pentatomomorpha, but only four or five in Enicocephalomorpha (Cobben, 1978; Schuh, 1979; Wheeler et al., 1993). The situation is unknown for all other taxa under consideration.

51. Number of tarsomeres in first-instar larvae: (0) one; (1) two.

The tarsi are 2-segmented in the first-instar larvae in Aphidoidea, Auchenorrhyncha, Coleorrhyncha, and Pentatomomorpha. Only one tarsomere is present in Enicocephalomorpha and Dipsocoromorpha (Cobben, 1978; Schuh, 1979; Wheeler et al., 1993). The situation is unknown for the other taxa under consideration.

52. Forewings: (0) completely uniform or if differentiated, not forming a distinct corium-clavus and membrane; (1) forewing divided into a distinct corium-clavus and membrane.

The forewing is divided into a distinct corium-clavus and membrane in all Heteroptera with the exception of Enicocephalomorpha. The character was coded as (–) for wingless taxa (Wheeler et al., 1993).

53. Cephalic trichobothria: (0) absent in adults; (1) present in adults.

Cephalic trichobothria are generally present in adults of Heteroptera (Wheeler et al., 1993) and Coleorrhyncha. They are absent in all other groups under consideration (e.g. Weber, 1969; Hamilton, 1981; Beutel and Weide, 2005; Yoshizawa, 2005).

54. Metathoracic scent gland system: (0) absent; (1) present.

A metathoracic scent gland system is present in Heteroptera (Wheeler et al., 1993; Schuh et al., 2009). It is absent in all other groups under consideration. It was proposed as a synapomorphy of Heteroptera and Coleorrhyncha by Kristensen (1981). However, the examination of a histological cross section series of *Hackeriella veitchi* revealed no metathoracic scent glands.

55. Labium: (0) not tubular; (1) tubular labium with three segments; (2) tubular labium with four segments.

The labium is tube-shaped and 4-segmented in Heteroptera (Stys, 1983) and Coleorrhyncha. It is 3-segmented in the majority of Reduviidae (Weirauch, 2008). A tubular labium with three segments is present in Auchenorrhyncha, Aphidoidea (Matsuda, 1965; Wheeler et al., 1993) and Psylloidea (Weber, 1928). It is not tubular in taxa with biting mouthparts. The situation is unknown for Rhynchophthirina, Anoplura, Aleyrodidae, and Coccoidea.

56. Insertion of tubular labium: (0) posteriorly on the head, (1) anteriorly on the head.

The tubular labium is inserted anteriorly on the head in Heteroptera, whereas it inserts posteriorly on the head in Sternorrhyncha, Auchenorrhyncha, and Coleorrhyncha (Carver et al., 1991; Wheeler et al., 1993; Weirauch and Schuh, 2011). The character was coded as (–) for taxa without a tubular labium. The situation is unknown for Anoplura and Rhynchophthirina (coded ?).

57. Dorsal abdominal glands in immature stages: (0) absent; (1) present.

Dorsal abdominal glands associated with the tergites are usually present in nymphs of Heteroptera (Weirauch, 2006; Weirauch and Schuh, 2011) and probably an autapomorphy for this group. They are absent in all other groups under consideration. However, we did not have nymphs of *Systelloderes* or *Corixidea*, therefore the character was coded as "?" for those groups.

58. Number of antennal flagellomeres: (0) more than 4, (1) 4 or less.

The number of antennal flagellomeres is reduced in Heteroptera and Coleorrhyncha (Weirauch and Schuh, 2011). Only one flagellomere is present in Coleorrhyncha. Two flagellomeres are present in Enicocephalomorpha and Dipsocoromorpha, and three in all examined Pentatomomorpha. The true lice have three or less flagellomeres and Odonata 2–4. All other examined species have multisegmented antennae.

59. Articulations between the mesomere, anterodorsal extension of ventral plate and posterior end of basal plate: (0) absent; (1) present.

In the male genitalia of Troctomorpha, Amblycera, and Ischnocera (Yoshizawa and Johnson, 2006) an articulation is present between the mesomere, the anterodorsal extension of the ventral plate, and the posterior end of the basal plate. It is absent in Psocomorpha, Rhynchophthirina, and Anoplura. The situation in the other taxa under consideration is unclear.

60. Length of basal apodeme of the phallic organ: (0) short; (1) long, longer than basal plate.

The basal apodeme is longer than the basal plate in Amblycera. It is short in Troctomorpha, Psocomorpha, and the remaining true lice (Yoshizawa and Johnson, 2006). The situation is unknown for the other taxa under consideration. It was coded as (–) for taxa without a basal apodeme.

61. Third posterodorsal corner of basal plate: (0) not extended; (1) extended posteriorly.

The third posterodorsal corner of the basal plate is extended posteriorly in Amblycera, whereas it is unmodified in the remaining true lice, in Psocomorpha and in Troctomorpha (Yoshizawa and Johnson, 2006). The situation is unknown for the other taxa under consideration.

62. Basal apodeme of the phallic organ: (0) present; (1) absent.

The basal apodeme is present in Troctomorpha and in Psocomorpha. It is also present in all true lice (Yoshizawa and Johnson, 2006). The situation is unknown for the other taxa under consideration.

63. Width of basal apodeme: (0) narrow; (1) as broad as or broader than basal plate.

The basal apodeme is broad in Ischnocera, Rhynchophthirina, and Anoplura, but narrow in Amblycera, Troctomorpha, and Psocomorpha (Yoshizawa and Johnson, 2006). The situation is unknown for the other taxa under consideration.

64. Ventral plates 1: (0) separated; (1) partly fused anteriorly.

The ventral plates 1 are partly fused anteriorly in Ischnocera, Rhynchophthirina, and Anoplura. They are separated in Amblycera, Troctomorpha, and Psocomorpha (Yoshizawa and Johnson, 2006). The situation in other taxa under consideration is unclear.

65. Ventral plates 2: (0) separated or partly fused; (1) completely fused.

The ventral plates 2 are completely fused in Ischnocera. In the remaining Phthiraptera, Psocomorpha, and Troctomorpha they are separated or only partly fused (Yoshizawa and Johnson, 2006). The situation in other taxa under consideration is unclear.

66. Mesomere of the aedeagus: (0) rounded posteriorly; (1) pointed posteriorly.

The mesomere is pointed posteriorly in Anoplura, Rhynchophthirina, and some Psocomorpha (coded as 1 for *Caecilius* sp.). It is rounded posteriorly in Ischnocera, Amblycera, and Troctomorpha (Yoshizawa and Johnson, 2006). The situation is unknown for the other taxa under consideration.

67. Posteromedian part of basal plate: (0) membranous; (1) sclerotized.

The posteromedian part of the basal plate is sclerotized in Anoplura and Rhynchophthirina. It is membranous in Ischnocera, Amblycera, Psocomorpha, and Troctomorpha (Yoshizawa and Johnson, 2006). The situation is unknown for the other taxa under consideration.

68. Anterior end of mesomere: (0) articulated with basal plate; (1) articulated with paramere.

The anterior end of the mesomere is articulated with the paramere in Anoplura. In the remaining true lice, in Psocomorpha and Trocto-

morpha it is articulated with the basal plate (Yoshizawa and Johnson, 2006). The situation in the other taxa under consideration is unclear.

69. Paired ocelli in nymphs or larvae: (0) absent; (1) present.

Paired larval ocelli are absent in Holometabola (Beutel et al., 2011) and they are also missing in acercarian nymphs. The situation is unknown for nymphs of Coleorrhyncha (coded ?).

70. Intrinsic antennal muscles (Mm. scapopedicellares) in immature stages: (0) absent; (1) present.

The Mm. scapopedicellares are absent in holometabolan larvae (Beutel et al., 2011). The muscle is present in immature stages of all other taxa under consideration. The situation is unknown for nymphs of Coleorrhyncha (coded ?).

71. Ventral metasternal process: (0) absent; (1) present.

The ventral metasternal process is present in Holometabola (Beutel et al., 2011). It is generally absent in the other groups under consideration.

72. Sensory plate organs of pedicel: (0) absent; (1) present.

A sensory plate organ of the pedicel is present in Fulgoromorpha (Bourgoin, 1985). It is absent in all other groups under consideration.

73. Evan's organ: (0) absent, (1) present.

Evan's organ, a sensory pit on the maxillary plate, is present in Auchenorrhyncha and Coleorrhyncha. It is absent in Sternorrhyncha and Heteroptera (Evans, 1973; Bourgoin, 1986). The character was coded as (–) for taxa without a maxillary plate, i.e. those with biting mouthparts. The situation is unknown for Anoplura (coded ?).

74. Ductus ejaculatorius: (0) normal; (1) modified as a sperm pump.

The ductus ejaculatorius is modified as a sperm pump in Psylloidea and Aleyrodidae (Schlee, 1969).

75. Proximal abdomen pediculate by reduction of the 1st and 2nd segment: (0) absent; (1) present.

The proximal abdomen is pronouncedly narrowed by the distinct reduction of the 1st and 2nd segment in Psylloidea and Aleyrodidae (Schlee, 1969). This is not the case in all other examined species.

76. Hind coxae: (0) normally developed; (1) broad, closely adjacent.

The hind coxae are broadened and medially closely adjacent in Psylloidea and Aleyrodidae (Schlee, 1969). This is not the case in all other taxa under consideration.

77. Proboscis: (0) absent; (1) shifted posteriorly between bases of procoxae; (2) not shifted posteriorly between bases of procoxae.

Mouthparts forming a proboscis are present in Hemiptera, Rhynchophthirina, Anoplura, and Thysanoptera. The proboscis is shifted posteriorly and placed between the bases of the procoxae in Sternorrhyncha (Hamilton, 1981).

78. Posterior parts of the head capsule: (0) sclerotized; (1) membraneous.

The posterior parts of the head capsule are membranous in Sternorrhyncha (Hamilton, 1981). This is not the case in all other taxa under consideration.

79. Connective tissue occluding occipital foramen: (0) absent; (1) present.

The foramen occipitale is occluded by connective tissue in Ischnocera, Rhynchophthirina, and Anoplura (Lyal, 1985). This is not the case in all other taxa under consideration.

80. Ovipositor simplified: (0) absent; (1) present.

A simplified ovipositor was proposed as an autapomorphy for Psocodea by Grimaldi and Engel (2005a,b).However, it is normally developed in Psocoptera (Yoshizawa, 2002) and only reduced in Phthiraptera (Boudreaux, 1979; Lyal, 1985). It is also simplified in members of the Enicocephalidae (Wygodzinsky and Schmidt, 1991).

81. Spiracular glands: (0) absent; (1) present.

Spiracular glands are present in Ischnocera, Anoplura, and Rhynchophthirina (Lyal, 1985). They are absent in all other groups under consideration.

82. Extension of the occipital apodeme reaching into the thorax: (0) absent; (1) present.

The occipital apodeme extends into the thorax in Ischnocera, Anoplura, and Rhynchophthirina (Lyal, 1985).

83. Pronotum and procoxae: (0) not fused; (1) fused.

The pronotum is fused with the procoxae in Anoplura and Rhynchophthirina (Piotrowski, 1992). This is not the case in all other groups under consideration.

84. Position of anterior tentorial pits: (0) frontal side of head; (1) absent; (2) shifted dorsally.

The anterior tentorial pits are present in Hemiptera, Thysanoptera, and all outgroups. They are absent in Anoplura and Rhynchophthirina. They are shifted to the dorsal side of the head in Thysanoptera and Hemiptera (Hamilton, 1981). The situation is unknown for Coccoidea and Dipsocoromorpha (coded ?).

85. Fusion of head and thorax: (0) absent; (1) present.

The head is fused with the thorax in Coccoidea (Hamilton, 1981). It is connected with the head by the cervical membrane in all other examined species.

86. Body and head: (0) not flattened; (1) dorsoventrally flattened.

The head and body are distinctly flattened dorsoventrally in Troctomorpha, Phthiraptera, Coccoidea, and Coleorrhyncha.

87. Hind femora: (0) not enlarged; (1) enlarged.

The hind femora are distinctly enlarged in Orthoptera, Troctomorpha, Amblycera, and Ischnocera. Grimaldi and Engel (2005a,b) claimed enlarged hind femora as a synapomorphy of all Phthiraptera, but the hind femora of Pediculidae (Anoplura) and Rhynchophthirina are normally-sized.

88. Meso- and metanotum: (0) not fused; (1) fused.

The meso- and metanotum are fused in Troctomorpha and true lice (Lyal, 1985). They are not fused in all other groups under consideration.

89. Compound eyes: (0) not reduced; (1) only 2 ommatidia or less.

The eyes are reduced in Troctomorpha, Phthiraptera (Lyal, 1985) and Coccoidea. They are more or less well developed in all other groups under consideration.

90. Labial palp: (0) present; (1) absent.

The labial palps are absent in Acercaria (e.g. Hamilton, 1981; Lyal, 1985) with the exception of Thysanoptera.

91. Complex tymbal acoustic system: absent (0); present.

A complex tymbal acoustic system is present in Auchenorrhyncha (e.g. Claridge, 1985). It is absent in all other groups under consideration.

92. Aristate antennal flagellum: (0) absent; (1) present.

An aristate antennal flagellum is present in Auchenorrhyncha and Odonata (Mathur and Mathur, 1961).

93. Malpighian tubules: (0) more than six; (1) six; (2) four or less.

Six Malpighian tubules are present in Zoraptera whereas only four are present in Acercaria (e.g. Wigglesworth and Salpeter, 1962; Kristensen, 1981; Lyal, 1985). Between eight and four are usually present in Holometabola (Beutel et al., 2011)but the number is distinctly higher in *Macroxyela* and other hymenopterans.

94. Labrum: (0) not narrowed; (1) narrowed.

The labrum is distinctly narrowed in Hemiptera and Thysanoptera (Hamilton, 1981).

95. Mandibular and lacinial stylets: (1) unicondylar; (0) dicondylar.

The mandibular and lacinial stylets are unicondylar in Hemiptera and Thysanoptera (Mickoleit, 1963; Hamilton, 1981). The character was coded as (–) for groups without piercing-sucking mouthparts.

96. Pedunculate eggs (with stalk): (0) absent; (1) present.

Pedunculate eggs are produced in Psylloidea and Aleyrodidae (Carver et al., 1991), and similar conditions occur in some species of

Aphidoidea (Quednau and Martin, 2006) and Hymenoptera [Tenthredinidae (Boeve, 1991)]. The eggs are not pedunculate in all other groups under consideration. The situation is unknown for Troctomorpha (coded ?).

97. Gut with filter chamber containing Malpighian tubules: (0) absent; (1) present.

A filter chamber containing Malpighian tubules is present in the digestive tract of Cicadomorpha (Evans, 1963). This is not the case in the other taxa under consideration (e.g. Albrecht, 1953; Evans, 1963; Rhodes et al., 1997). The situation is unknown for Enicocephalomorpha, Dipsocoromorpha, and Psocoptera.

98. Coronal (= median epicranial) suture: (0) absent; (1) present.

A coronal suture is absent in Hymenoptera (Beutel and Vilhelmsen, 2007), Zoraptera (Beutel and Weide, 2005), Odonata (Mathur and Mathur, 1961) and Thysanoptera. It is present in all other groups under consideration, even though it is only weakly developed in *Liposcelis* sp. (Troctomorpha, Hamilton, 1981).

99. Parempodia on unguitractor plate: (0) absent; (1) elongate and setiform, inserted in an alveolus.

A pair of setiform parempodia inserted in an alveolus is present on the distal part of the unguitractor plate in Pentatomomorpha. In Psylloidea, Enicocephalomorpha, and Dipsocoromorpha the parempodia are present, but the alveolus is not as distinct as in Pentatomomorpha.

100. Number of tarsal segments: (0) one; (1) two; (2) three; (3) more than three.

Only one tarsal segment is present in Ischnocera, Rhynchophthirina, Anoplura, Coccoidea, and Thysanoptera partim [coded as 0/1 (Beutel and Gorb, 2001)]. Two segments are present in *Zorotypus* (Beutel and Weide, 2005), Trogiomorpha, Psocomorpha, Amblycera, Psylloidea, Aleyrodidae, Aphidoidea, and Coleorrhyncha. Three tarsal segments are present in Plecoptera, *Embiopsocus* sp. (Troctomorpha), all investigated Auchenorrhyncha, and Heteroptera. More than three tarsal segments are present in Odonata, *Nevrorthus* sp. and *Xyela* sp. (Hymenoptera).

101. Arolium: (0) absent; (1) present; (2) eversible; (3) bilobed.

A vesicle-like, eversible arolium is in present in Thysanoptera, and is apparently an autapomorphy for this group. A largely unmodified arolium is present in Cercopidae, Cixiidae, Delphacidae, and Fulgoridae, and also in Coleorrhyncha and males of *Corixidea* (Dipsocoromorpha). The arolium is also eversible in Fulgoridae to a certain degree (Frantsevich et al., 2008), but not as strongly as in Thysanoptera. In Membracidae, and Cicadellidae the arolium is distinctly bilobed; the same condition is found in *Psylla* sp. An arolium is absent from *Zorotypus*, Psocodea, Aphidoidea, Coccoidea, Cicadidae, and the remaining Heteroptera.

Beutel and Gorb (2001) claimed that an arolium is present in Miridae (Heteroptera), but this might be due to a misinterpretation. For *Lygus hesperus* (Miridae), for instance, Shrestha et al. (2007)disregards the common nomenclature and refers to the attachment structures as an arolium. However, his figures show clearly that these paired structures are in fact pulvilli.

102. Sticky terminal lip ofarolium: (0) absent; (1) present.

In Fulgoromorpha a sticky terminal lip can be distinguished from the rest of the arolium. This sticky lip is the part of the arolium that actually is in contact with the surface. In Psylloidea the terminal part of the bilobed arolium is also morphologically different from the rest. The character was coded as (–) for taxa without arolium.

103. Pulvilli: (0) absent; (1) present.

Pulvilli are present in Trogiomorpha, Psocomorpha, Coccoidea, and Pentatomomorpha. They are fleshy in *Drepanosiphum* sp., but reduced to thin hair-like structures in other aphids.

In Coccoidea the paired fleshy structures at the inner base of the claws are almost always referred to as claw digitules (Cockerell, 1893; Kondo, 2006). However, it appears very likely that they are in fact pulvilli.

104. Euplantulae: (0) absent, (1) present.

Euplantulae are present on the tarsal segments in Orthoptera, *Xyela* sp. (Hymenoptera),*Trinoton* sp. (Ambylcera) and in Anoplura. The euplantulae in *Trinoton* sp. are flat and shovel-shaped, whereas they are pad-like in *Xyela* sp. and Orthoptera.

105. Number of claws: (0) one; (1) two; (2) reduced into spoon-shaped plates; (3) main claw plus accessory claw.

Only one claw is present in Coccoidea and in Pediculidae (Anoplura). In Rhynchophthirina there is a main claw plus a smaller accessory claw. In all other examined species there are two claws. In Thysanoptera the claws are reduced into spoon-shaped, laterally ensheathing plates.

106. Claw teeth: (0) absent; (1) present.

Distinct claw teeth are present in Odonata, *Xyela* sp. (Hymenoptera), Neuroptera, and *Embiopsocus* sp.

107. Protuberance with microtrichia on distolateral side of the pretarsus: (0) absent; (1) present.

A protrusion covered with microtrichia is present on the ventral side of the pretarsus in Cercopidae, on the distolateral region of the arolium, directly below the claws. This condition not found in any other groups is probably an autapomorphy of the family. The character was coded as (–) for taxa without arolii.

108. Sensorial setae on mesal side of arolium: (0) absent; (1) present.

On the distal part of the bilobed arolium of Membracidae and Cicadellidae there are two thin sensillae. Neither on the bilobed arolium of Psylloidea nor on the arolii of the other examined species are such sensilla present.

109. Adhesive claw setae: (0) absent; (1) present.

A thin seta arising from the claws directly distad each pulvillus is present in Trogiomorpha.

110. Eversible structure between tibia and tarsus: (0) absent; (1) present.

An eversible pad between the tibia and tarsus is present in *Macrosiphum* sp. (Aphidoidea). The species with this structure lack fleshy pulvilli (and *vice versa*).

111. Tibial thumb-like process: (0) absent; (1) present.

A tibial thumb-like process (Soler-Cruz and Martin-Mateo, 2009) is present on the ventral side of the apex of the tibia of Pediculidae. A sclerotized spine on the apex of the "thumb" forms a pincer together with the claw.

112. Empodial Paronychium: (0) absent; (1) present.

A thin lobe-like structure arises between the claws in Aleyrodidae. Most authors follow Quaintance and Baker (1913) in referring to this structure as "paronychium". It's still questionable if the structure is homologous with an arolium or an empodium. Further study of the internal fine structure is required.

113. Tarsal apophysis on the ventral side of the tarsus: (0) absent; (1) present.

A spiny tarsal apophysis (= lamella (Keilin and Nuttall, 1930) is present on the ventral side of the tarsus of the head louse and the body louse. This apophysis is placed on a cushion-like structure not mentioned by Soler-Cruz and Martin-Mateo (2009).

114. Two dorsal capitate setae: (0) absent; (1) present.

In Coccoidea two capitate setae [= tarsal digitules after (Cockerell, 1893)] are present on the dorsal side of the pretarsus.

115. Flag-like sensilla on the 1st tarsal segment: (0) absent; (1) present.

Two flag-like sensilla are present on the ventral side of the first tarsal segment of *Trinoton* sp. (Amblycera). Sensilla of this type were not found in any other examined species.

116. Fingerlike process below claw: (0) absent; (1) present.

A fingerlike process beneath the claw is present in *Pediculus humanus capitis*. It has probably a sensory function (proprioreceptor), providing information about the opening angle of the claws (Soler-Cruz and Martin-Mateo, 2009). A very similar structure is present in the body louse (*Pediculus humanis corporis*).

117. Ventral brush: (0) absent; (1) present.

The ventral distal rim of the distal tarsomere of Enicocephalomorpha and Pentatomomorpha bears a row of microtrichia (= ventral brush after Weirauch, 2005). The ventral brush is absent in Dipsocoromorpha and all other taxa under consideration.

Characters not included in the matrix

118. Clasping mechanism with tibia and tarsus of foreleg opposable to apex of femur: (0) absent; (1) present.

The forelegs are uniquely modified as raptorial legs in Enicocephalomorpha (Stys, 1983; Wheeler et al., 1993).

119. Head shape: (0) not conspicuously constricted behind compound eyes, ocelli not located on posterior lobe of head; (1) conspicuously constricted behind compound eyes, ocelli located on posterior lobe.

The head of Enicocephalomorpha is distinctly constricted behind the compound eyes with the ocelli located on a posterior lobe of the head capsule. This is apparently an autapomorphy of the group (Stys, 1983; Wheeler et al., 1993).

120. Forewing-body coupling mechanisms: (0) not developed; (1) coupling mechanisms with push-button system and scutellar frena well developed.

The forewing-body coupling mechanism is present in Pentatomomorpha (Wheeler et al., 1993). The character was coded as (–) for wingless taxa.

121. Adhesive pads on metacoxae: (0) absent; (1) present.

According to Stys (1983) Dipsocoromorpha have adhesive pads on the metacoxae. However, no pads were found in *Ceratocombus australiensis* (Dipsocoromorpha).

3.1 Study II

Friedemann K, Beutel RG (in press)
Morphology of arolia in Auchenorrhyncha (Insecta, Hemiptera)
Journal of Morphology, DOI 10.1002/jmor.20290

Abstract: The pretarsal arolium serves as an attachment device in many groups of insects, enabling them to walk efficiently on smooth surfaces, where claws alone do not provide sufficient foothold. The arolia of representatives of all major lineages of Auchenorrhyncha are described and illustrated, mainly using scanning electron microscopy and histology. Glands inside the lumen of the arolia are described for the first time in this group. It is shown that the morphology of arolia within Auchenorrhyncha differs considerably. Some of them are even distinctly bilobed. The cuticle of the contact zone is thickened and formed of branching chitinous rods. In some cases two layers of rods oriented in different directions were found.

Significance in the present thesis: This study is focused on the acercarian order Auchenorrhyncha. In this group several different kinds of arolia are present. For the first time a gland inside the arolia of this lineage is described. A refined definition of arolium is proposed. It is shown that the bilobed structures of Membracoidea are clearly homologous to arolia.

Own contribution: 90%

Morphology of Arolia in Auchenorrhyncha (Insecta, Hemiptera)

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ABSTRACT The pretarsal arolium serves as an attachment device in many groups of insects, enabling them to walk efficiently on smooth surfaces, where claws alone do not provide sufficient foothold. The arolia of representatives of all major lineages of Auchenorrhyncha are described and illustrated, mainly using scanning electron microscopy and histology. Glands inside the lumen of the arolia are described for the first time in this group. It is shown that the morphology of arolia within Auchenorrhyncha differs considerably. Some of them are even distinctly bilobed. The cuticle of the contact zone is thickened and formed of branching chitinous rods. In some cases, two layers of rods oriented in different directions were found. An extended definition of "arolium" is proposed. J. Morphol. 000:000–000, 2014. © 2014 Wiley Periodicals, Inc.

KEY WORDS: evolution; attachment devices; cicada; pretarsus; glands

INTRODUCTION

The Auchenorrhyncha (cicada, plant hoppers, tree hoppers etc.) are a successful and species rich subgroup of Hemiptera. About 42,000 species are presently described and virtually all of them feed on plant sap. Living and walking efficiently on plant surfaces requires appropriate attachment structures. The main role in this context plays the arolium which is present in almost all groups of Auchenorrhyncha. The pretarsal arolium serves as attachment device in many groups of insects, enabling them to walk efficiently on smooth (and even vertical) surfaces, where claws alone do not provide sufficient foothold (Gorb and Beutel, 2001; Endlein and Federle, 2008).

It is assumed that liquid produced in a so-called arolium gland (=tarsal gland after Jarau et al., 2005) leaves oily footprints. The location of the gland differs. Although its usual site is the pretarsus or the arolium itself in hemimetabolous insects, it is usually located in the distal tarsal segment in holometabolan groups (Federle et al., 2001; Billen, 2009). With the footprint secretions, bees and ants mark the nest entrance or food sources (e.g., Butler et al., 1969; Schmitt et al., 1991). In addition to this specialized function in eusocial hymenopterans, Federle et al. (2001) pointed out that the secretion may often play a role in the context of attachment and locomotion on plant surfaces. It was shown that in some species the secretion is not released externally, but remains in the lumen of the arolium. During walking on smooth surfaces, the secretion fills and unfolds the arolium to increase the contact area between the adhesive pad and the substrate.

An arolium is per definition (Dashman, 1953) a median hollow lobe between the claws. It can be completely membranous or partly sclerotized (Beutel and Gorb, 2001). Its surface is almost always smooth and its cuticle consists of rod-like chitin crystallites oriented perpendicular or at some angle to the surface.

The presence of an arolium is a common feature in Neoptera and arguably belongs to the groundplan of this lineage which comprises about 98% of all known insect species. It occurs in many lineages: Plecoptera (Beutel and Gorb, 2001), several groups of Polyneoptera (see e.g., Rentz, 1991; Beutel and Gorb, 2008), Acercaria (see Friedemann et al., 2014), and Holometabola (Hennig, 1973; Nielsen and Common, 1991; Federle et al., 2001). The shape, armature, and complexity of the arolium differ considerably between taxa, and the homology of sclerites in holometabolan and non-holometabolan insects is still unclear.

The monophyly of Auchenorrhyncha is suggested by characteristic features, such as an aristate antenna, a complex tymbal acoustic system (Grimaldi and Engel, 2005), and a unique structure of the wing base (Yoshizawa and Saigusa, 2001). Even though these characters combined

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appear as convincing evidence, the monophyly of the order was repeatedly questioned during the last few years. A sistergroup relationship between Fulgoromorpha (planthoppers) and Heteroptera (true bugs) was proposed by von Dohlen and Moran (1995) in a study that was based on a single gene (18S rRNA) and a very limited taxon sampling. Hamilton (1981) suggested Sternorrhyncha as the sistergroup to Cicadomorpha (leafhoppers, treehoppers, spittlebugs, and cicadas). Recently, a sistergroup relationship between Cicadomorpha and Heteroptera appears as a serious alternative to the monophyly of Auchenorrhyncha. This was suggested in a study using combined paleontological, molecular, and morphological data (Bourgoin and Campbell, 2002), and similar results were obtained in recent studies based on transcriptomes (Letsch et al., 2012) and mitochondrial genomes (Cui et al., 2013), respectively. In the latter study, Fulgoromorpha were placed as the sistergroup of Coleorrhyncha, implying the paraphyly of Prosorrhyncha (=Heteropterodea). The possible paraphyly of Auchenorrhyncha raises the question whether the arolia of Fulgoromorpha and Cicadomorpha are homologous or whether they have evolved independently in both groups. Another question is the homology of the bilobed pads in Membracoidea. Because they are paired structures, they appear closer to the definition of pulvilli, which are paired adhesive pads usually inserted below the claws. However, they might also represent a strongly modified arolium.

These unsolved questions underline the necessity to study the structures in greater detail. The morphology of the arolium including internal fine structures has been rarely investigated. Slifer (1950) presented some drawings of the inner structures of the arolium of *Melanoplus differentialis* (Orthoptera, Caelifera). Cross sections showing internal details of the arolium of several roach species can be found in a study focused on the climbing ability of these insects (Roth and Willis, 1952). A description of the chitinous rods forming the distal part of the arolium in *Carausius morosus* (Phasmatodea) can be found in Bennemann et al. (2011). A detailed description of the arolium and arolium gland is only available for Mantophasmatodea (Eberhard et al., 2009), some members of Hymenoptera, such as bees and ants (e.g., Federle et al., 2001), and the stingless bee *Melipona seminigra* (Jarau et al., 2005). The arolium of members of Auchenorrhyncha has received very little attention so far. The structure of the chitinous rods forming the wall of the arolium in *Cercopis vulnerata* was illustrated and described in Beutel and Gorb (2001) and some fragmentary descriptions of the outer morphology and sclerites of the pretarsi in Fulgoroidea (Fennah, 1945; Doering, 1956) are available. Frantsevich et al. (2008) focused on the functional aspects of the arolium in lantern-flies (Fulgoridae). He described the chitinous rods in the arolium but did not mention any glands even though lantern-flies leave visible footprints on glass-slides.

In this study, we present the first description of an arolium gland in Auchenorrhyncha and the first three-dimensional (3D)-reconstruction of this structure and of the other elements of the pretarsus. The outer and inner morphology of the pretarsi in the major auchenorrhynchan lineages are described, compared, and discussed in the context of current phylogenetic hypotheses.

MATERIAL AND METHODS

Examined Taxa

Unless indicated otherwise, all specimens were collected in Germany, Thuringia and stored in 70% ethanol. Taxa were chosen so that each major lineage of Auchenorrhyncha is represented by at least one species. At least two specimens per species were examined.

Fulgoromorpha. Delphacidae: *Javasella* sp.; Cixiidae: *Cixius* sp.; Dictyopharidae: *Dictyophara europaea* (L., 1767).

Membracoidea. Cicadellidae: *Cicadella viridis* L., 1758; *Ledra aurita* L., 1758; Membracidae: *Centrotus cornutus* (L., 1758); *Strictocephala bisonia* Kopp and Yonke 1977 (Coll: Italy, Piemont); *Gargara genistae* (Fabricius, 1775).

Cicadoidea. Cicadidae: *Cicadetta montana* (Scopoli, 1772)

Cercopoidea. Aphrophoridae: *Philaenus spumarius* L., 1758; *Neophilaenus* sp. Cercopidae: *C. vulnerata* Rossi, 1807

Histological Sections

Specimens (six species, two insects per species) were embedded in Araldite CY 212® (Agar Scientific, Stansted/Essex, UK). Cross sectioning was carried out with a HM 360 microtome (Microm, Walldorf, Germany). The sections (thickness: 1 μm) were stained with Toluidin blue (Waldeck GmbH & Co., KG/Division Chroma, Münster, Germany) and documented with a PixeLINK PL-A622C digital camera (PixeLINK, Ottawa, Canada) at 20x magnification.

Scanning Electron Microscopy

For scanning electron microscopy (SEM; Philips XL30 ESEM) specimens were completely dehydrated with ethanol (100%) over several stages, dried using hexamethyldisilazane (HMDS; Brown, 1993), sputter-coated with gold (Emitech K500, Ashford, Kent, UK), and fixed on a rotatable specimen holder (Pohl, 2010). Scandium software (Soft Imaging System, Münster, Germany) was used for obtaining high-resolution images. To investigate the ultrastructure of arolia, specimens were embedded in methacrylate. Cross and longitudinal sectioning was carried out, cutting the middle region of the arolium. Methacrylate was then dissolved using Xylol. Xylol was replaced gradually by acetone and specimens were dried at the critical point and subsequently examined using the SEM.

3D-Reconstruction

Alignment and 3D-reconstructions based on histological cross section series were prepared with Mercury Amira® 4.1.2 (Mercury Computer Systems, Berlin, Germany). All slices were used (203 in total). Surfaces were smoothed with Maya® 7.0 (Autodesk GmbH, Munich, Germany). An interactive 3D-model of the tarsus of *P. spumarius* is provided in Figure 5.

Light Microscopy and Image Editing

External features were examined and drawn using a stereo microscope MZ 12.5 with a camera lucida (LEICA). Line

drawings were further processed with Adobe Illustrator®. All figures in this article were assembled in Adobe Photoshop® and labeled with Adobe Illustrator® (San Jose, CA).

RESULTS

Fulgoromorpha

The arolium consists of a membranous lobe detached from the claws. In the contact zone on the distal part of the arolium (cz, Fig. 1D), the thickness of the cuticle is increased from 0.7 to ~28 μm (tc, Fig. 3A). This thickened area extends over the entire width of the arolium. The cuticle in this area is formed by chitinous rods oriented at an angle to the surface (cr, Fig. 1B). The rods are thicker proximally, and branch into finer subunits closer to the surface of the pad. The dorsal part of the arolium is flanked by a pair of sclerotized plates (dpl, Fig. 2; =dorsolateral plates after Frantsevich et al., 2008). Four long bristles arise from the lateral side of the claws in *Dictyophara*. The number of bristles is variable; in *Javasella* and *Cixius* (Fig. 1J), only a single one is present. The base of the claws is covered with scale-like distally tapering microtrichia. The gland tissue in the arolium is well-developed (agl, Figs. 3A, 4B) and fills out most of the lumen. Its proximal part turns into the epidermis of the leg. The gland is not directly in contact with the thickened cuticle. It is folded and shaped like a horizontal S (agl, Fig. 4B). There are no muscles in the pretarsus.

Cercopoidea: Aphrophoridae and Cercopidae

The tarsi are three-segmented. The ventral side of the pretarsus of the examined species is medially distinctly incised (Fig. 1C,K). A protrusion with a vestiture of microtrichia (mt, Fig. 1A,C) is present on the distolateral region of the pretarsus, directly below the claws. A Y-shaped sclerite bearing 3–4 thick bristles on each arm (sb, Fig. 2A) is present on the ventral part of the pretarsus. Proximally, the sclerite merges with the unguitractor plate (ut, Fig. 2A). On the dorsal side, the claws enclose a large V-shaped sclerite (dpl, Fig. 2B,C). Three setae are inserted on each side of the distal part of the arms of the "V." The position of these setae varies between the species. A smaller, rectangular sclerite is present distally to the "V" in *Neophilaenus*. This sclerite is a thin band in *Philaenus* and *Cercopis* and much smaller. Apart from this, there are no differences in the sclerotization of the pretarsi of the examined species of Cercopidae and Aphrophoridae.

On the distal ventral side of the pretarsus, a heart-shaped (*Cercopis*, *Philaenus*) area with an extremely thickened cuticle is present (~31 μm; tc, Fig. 3B). This contact zone (cz, Fig. 1A,C) is free of any microtrichia or setae, whereas the membrane surrounding it (thickness only ca. 0.5 μm) is covered with small microtrichia. The cuticle in the contact zone is formed by branched rods oriented at an angle to the surface. The rods are thicker proximally, and branch into thinner subunits closer to the surface of the pad. The area of thickened cuticle is medially invaginated (Fig. 3B) in *Cercopis* and *Philaenus*. This is probably the resting position of the arolium. In *Neophilaenus*, the contact area is considerably inflated (Figs. 1A, 2C) and extends well over the claws. Additionally, on the distal part of the pretarsus, a second layer of rods is present (cr2, Figs. 3B, 4A). It originates from the arolium gland and connects it with the rods of the cuticle.

The arolium gland is well-developed (Figs. 3B, 4A) and fills out most of the lumen of the pretarsus (Fig. 5). This tissue is connected with the epidermis of the leg cuticle and is abruptly narrowed after entering the distal tarsus. In the pretarsus, the gland is composed of two layers. The dorsal layer is adjacent to the dorsal cuticle. Distally, it reaches downward to approximately half length of the pretarsus before connecting with the ventral layer, thus forming a sack-like structure with the opening to the tarsus. The ventral side of the pretarsus lacks an epidermis. There is no contact between the ventral gland layer and the thickened cuticle of the arolium. Only the secondary rods on the distal part of the gland are in contact with the thickened cuticle (Fig. 4A). No openings of the gland for the release of secretions were found.

Membracoidea: Cicadellidae, Membracidae

The tarsi are three-segmented. The proximal segment is the smallest. The surface of the dorsal side of the pretarsus appears scaly. The arolium (Figs. 1G,J, 2D,E) is distinctly bilobed and largely fused with the claws. Only the tip of the claws is free. The unguitractor plate has a scaly surface structure. In *L. aurita*, a small triangular sclerite (dpl, Fig. 2E) is present adjacent to the claws on the dorsal side of each lobe of the arolium. A trichoid sensillum is protruding from this structure. Close to the juncture of the two lobes, two small sclerites are inserted. They are absent in *Cicadella*.

In the examined membracid species (*C. cornutus*, *S. bisonia*, and *G. genistae*), the dorsolateral sclerites are much larger (Fig. 2D) and cover a large proportion of the dorsal side of the lobes of the arolium. On their distal part, each of the sclerites bears a trichoid sensillum. Sclerites are missing on the ventral side of the pretarsus in all examined species.

The cuticle in the contact zone is also thickened and formed by branched rods (cr, Figs. 1F,H, 3C). However, due to the bilobed arolium, an area of thickened cuticle is present on each lobe, respectively. The second layer of rods is present in both lobes and even more pronounced than in

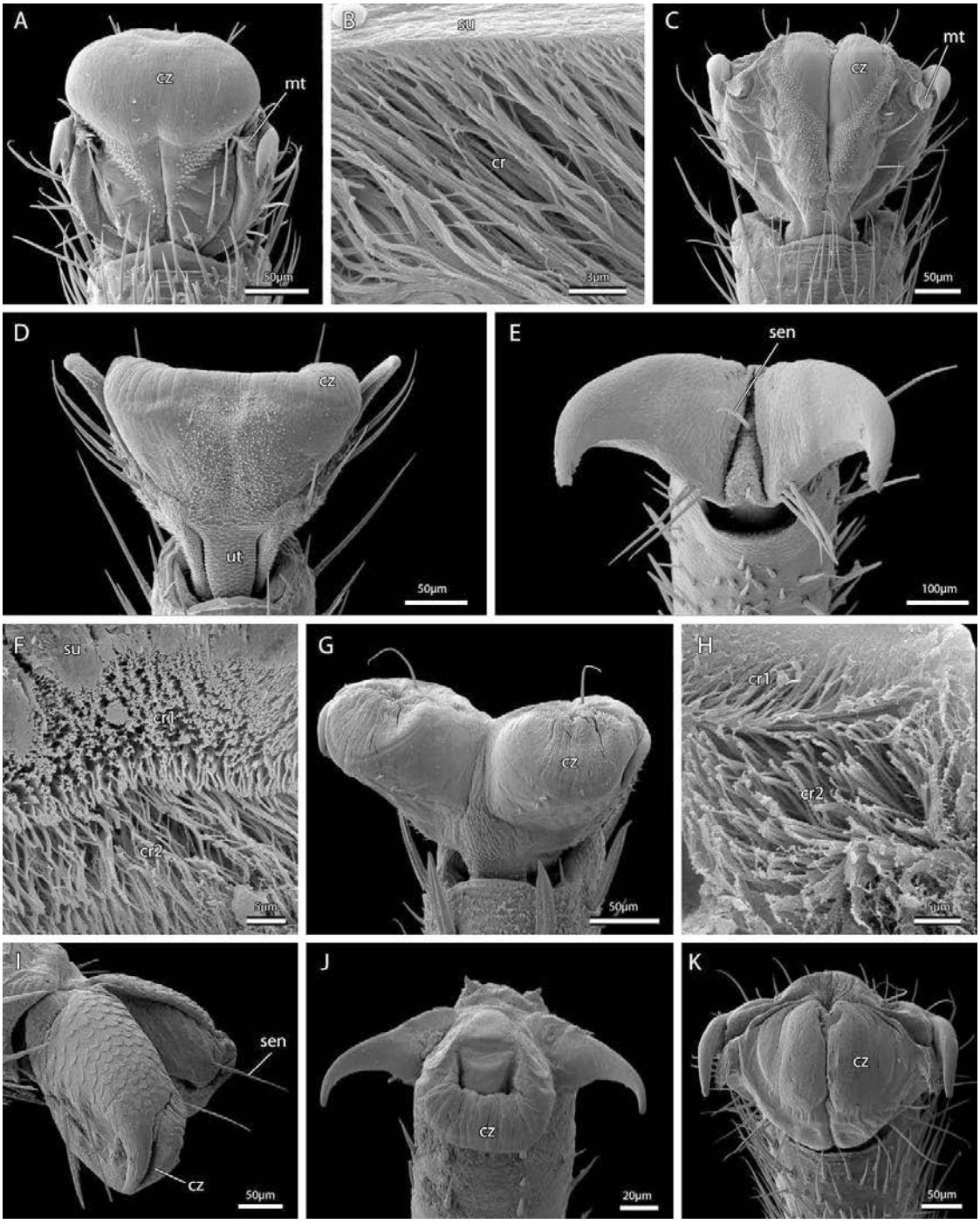

Fig. 1. Tarsi of Auchenorrhyncha, SEM images. (**A**) *Neophilaenus* sp., ventral view. (**B–C**) *Philaenus spumarius*, (B) cross section through the contact zone of the arolium and (C) ventral view of pretarsus. (**D**) *Dictyophara europaea*, ventral view. (**E**) *Cicadetta montana*, frontal view. (**F–H**) *Cicadella viridis*. (F) Cross section through the contact zone of the arolium. (G) Frontoventral view. (H) Longitudinal section through the contact zone of the arolium. (**I**) *Centrotus cornutus*, lateral view. (**J**) *Cixius* sp., frontal view. (**K**) *Cercopis vulnerata*, frontal view. Cr, chitinous rods; cr1, chitinous rods of the first, outer layer, cr2, chitinous rods of the second, inner layer; cz, contact zone; mt, bundle of microtrichia; su, outer surface of the arolium; ut, unguitractor plate.

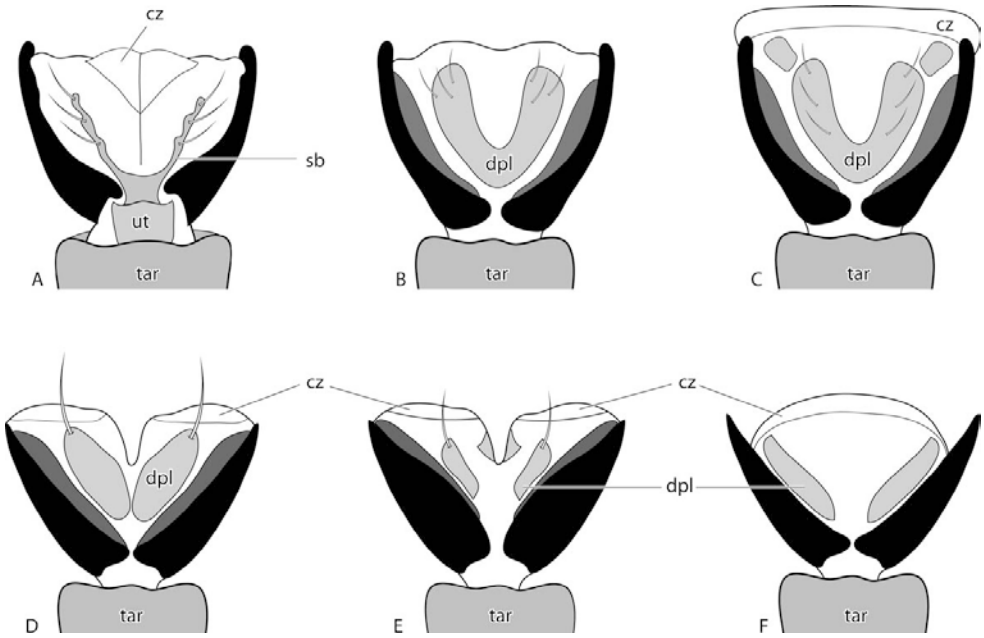

Fig. 2. Tarsi of Auchenorrhyncha, line drawings. Claws black, sclerotized parts gray, membranous parts white. (**A**) *Philaenus spumarius*, ventral view. (**B**) *Philaenus spumarius*, dorsal view. (**C**) *Neophilaenus* sp., dorsal view. (**D**) *Centrotus cornutus*, dorsal view. (**E**) *Ledra aurita*, dorsal view. (**F**) *Dictyophara europaea*, dorsal view. Cz, contact zone; dpl, dorsolateral plate; sb, sclerotized bar; tar, tarsus; ut, unguitractor plate.

Cercopidae. The rods of this layer are much thicker than the ones forming the other layer (cr1 and cr2, Fig. 1F,H). They do not extend through the entire layer of the contact zone, but only reach the middle region (Fig. 3C). Using SEM, it could be observed that the layers differ not only in thickness of the rods, but also in their direction (Fig. 1F,H). The inner layer is about 20-μm thick, and the outer one approximately 18 μm. The inner layer connects the well-developed arolium gland (agl, Fig. 3C) with the outer layer of the thickened cuticle.

Cicadoidea

In *Cicadetta* attachment structures are missing completely (Fig. 1E). Three thick bristles are present on the ventral base of the large claws. A single trichoid sensillum is inserted between the claws (sen, Fig. 1E).

DISCUSSION

Evolution

The monophyly of Auchenorrhyncha has been discussed controversially during the last years. It

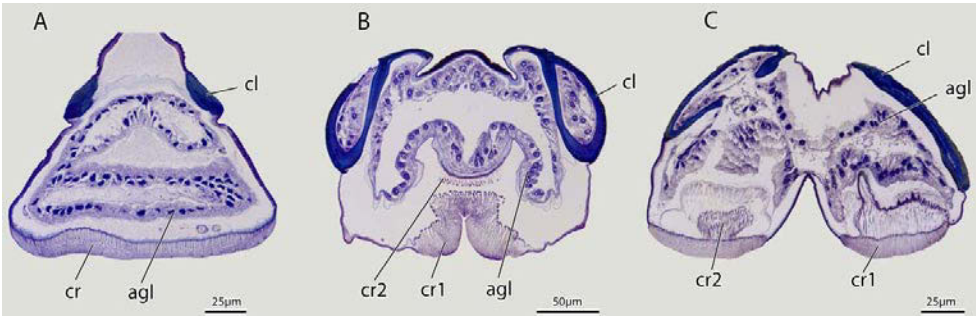

Fig. 3. Pretarsi of Auchenorrhyncha, histological cross sections. (**A**) *Dictyophara europaea*, (**B**) *Philaenus spumarius*, and (**C**) *Cicadella viridis*. Agl, arolium gland; cl, claws; cr, chitinous rods of the contact zone; cr1, outer layer of chitinous rods; cr2, inner layer of chitinous rods.

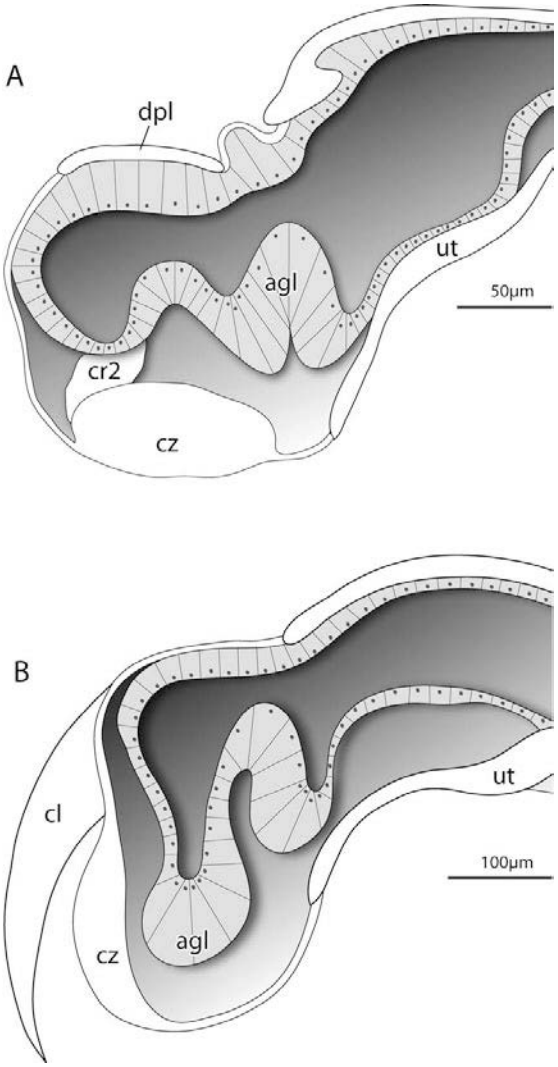

Fig. 4. Longitudinal sections of pretarsi of Auchenorrhyncha, line drawings. (**A**) *Philaenus spumarius* and (**B**) *Dictyophara europaea*. Agl, arolium gland; cl, claw; cz, contact zone; dpl, dorsolateral plate; cr2, second layer of chitinous rods; ut, unguitractor plate.

is supported by analyses of sequences of a broad array of genes (Urban and Cryan, 2007) and in an even more extensive study using seven gene regions (Cryan and Urban, 2012).

However, it was challenged in other studies. A sistergroup relationship between Cicadomorpha and Heteropterodea (Heteroptera + Coleorrhyncha) appears as a serious alternative to the monophyly of Auchenorrhyncha. This was suggested in an evaluation of combined paleontological, molecular, and morphological data (Bourgoin and Campbell, 2002) and is also suggested by the results of recent studies based on transcriptomes (Letsch et al., 2012; Letsch and Simon, 2013). However, the latter studies included only three members of Cicadomorpha, all of the same family (Cicadellidae). Due to this limited taxon sampling, the results of the analyses do not allow to draw conclusions on the evolution of cicadomorph attachment devices.

The conflicting hypotheses impede the evaluation of the character evolution in the case of attachment structures and other character systems. The concept of monophyletic Auchenorrhyncha, which appears presently best supported (e.g., Cryan and Urban, 2012), and the character state distribution suggest that a single-lobed arolium is a groundplan feature of this lineage. It is secondarily bilobed in Membracoidea, absent in Cicadoidea, and resurfaces in Cercopoidea as a single eversible lobe. The eversible arolium of the latter group is the most complex arolium within Auchenorrhyncha. A bilobed arolium evolved twice within Acercaria: in Membracoidea and in Psyllidae (Sternorrhyncha). In the potential sistergroup of Cicadomorpha, the Heteropterodea, a single-lobed arolium is present in Coleorrhyncha and some male members of Schizopteridae (Heteroptera, Dipsocoromorpha). For the morphology of the attachment devices of related groups (Sternorrhyncha, Psocodea, Thysanoptera, Coleorrhyncha, Heteroptera), see Friedemann et al. (2014).

Sclerites

The dorsal part of the examined arolia is usually more or less sclerotized. All sclerites on the pretarsi of the examined members of Auchenorrhyncha bear sensilla. Interestingly, the sockets of the setae of the ventral sclerite of *Philaenus* reach far into the arolium and are embedded in the gland tissue (Fig. 5, interactive 3D-reconstruction). Sclerites covering the dorsal surface of arolia (dorsolateral sclerites) occur also in other groups of insects, such as for instance Tipulomorpha (*Tipula hortulana*, Gladun et al., 2009). In *Tipula*, the dorsal plates act as extensors of the arolium. They maintain the narrow shape of the folded arolium like the cover spine of a book (Röder, 1986). While walking on smooth surfaces, the claws diverge and thereby flatten the extensor sclerites, which in turn expand the arolium and thus increase the contact area between the attachment pad and the surface (Gladun et al., 2009). It is unlikely that the dorsal sclerites in the examined Auchenorrhyncha have the same function. The bilobed arolium of Membracoidea probably lacks the potential to expand, as we did not find a single specimen in which the arolium was visibly inflated. The movement of the dorsal plates in Fulgoridea has been described in Frantsevich et al. (2008), but their role in everting the terminal part of the arolium is

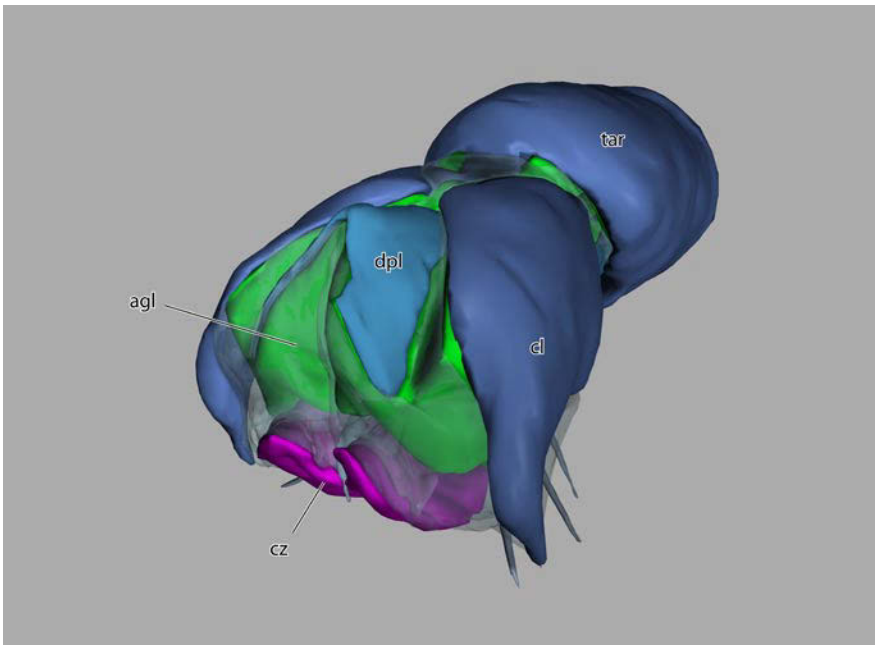

Fig. 5. Pretarsus of *Philaenus spumarius*, interactive 3-dimensional reconstruction. Sclerotized parts blue, membrane covering most of the pretarsus semitransparent, glands green, thickened cuticle of the contact zone pink. Agl, arolium gland; cl, claw; cz, contact zone; dpl, dorsal plate; tar, tarsal segment.

uncertain, as the pronation of the claws alone also stretches the base of the arolium.

Inflation and folding of the arolium appears possible in the examined Aphrophoridae. The contact area of the arolium of *Philaenus* was strongly invaginated medially (Fig. 1C), which likely is the resting position. However, we did not find an expanded arolium in any of the specimens at our disposal. In contrast, the contact area was inflated and extended well beyond the claws in specimens of *Neophilaenus* sp., which belong to the same family. This is apparently not an artifact as this condition was also observed in living specimens. However, the position of the dorsolateral sclerites was the same in the deflated and inflated condition, which clearly suggests that these sclerites are not involved in inflating the arolium.

Thickened Cuticle

The cuticle of the contact zone is generally distinctly thickened in insects with arolia (Fulgoromorpha: Frantsevich et al. 2008; Blattodea: Roth and Willis, 1952; Orthoptera: Slifer, 1950; Mantophasmatodea: Eberhard et al., 2009; Phasmatodea: Scholz, 2009; Bennemann et al., 2011; Hymenoptera: Federle et al., 2001). It is usually formed by chitinous rods in different attachment devices (e.g., Beutel and Gorb, 2001) but the arrangement of these structural elements varies. Some of them are dendritic structures (Auchenorrhyncha), whereas cross-connections between them occur in some species (Euplantulae of *Tettigonia*, Perez Goodwyn et al., 2006). In Orthoptera, the pads might function as a damper during jumping and landing (Perez Goodwyn et al., 2006). The rods stabilize the shape of the pad to prevent strong deformation. A similar function can be assumed for Auchenorrhyncha which are almost generally characterized by a good jumping capacity (e.g., *Cercopis vulnerata*: Gorb, 2004; *P. spumarius*: Burrows, 2006; *C. viridis*: O'Brien, 2002; Bonsignori et al., 2012: Fulgoromorpha). Interestingly, the only nonjumping species of our taxon sampling lacks the arolium, which suggests a correlation between these features. The architecture of the rods may also help to adapt to irregularities of the substrate surface.

A second layer of chitinous rods is described here for the first time. This additional layer is present in the investigated members of Cercopoidea and Membracoidea, but not in Fulgoromorpha. It probably is a potential apomorphy of Cicadomorpha.

Glands

A gland within the arolium is present in all examined members of Auchenorrhyncha. A gland directly located within the arolium has only been described for Mantophasmatodea (Eberhard et al.,

2009). However, it is conceivable that it is more common than previously thought. In cockroaches (Roth and Willis, 1952) and grasshoppers (Slifer, 1950), gland-like tissue is visible in histological sections of the pretarsus, but it was referred to as epidermis in these studies. As most cockroaches leave visible footprints on glass-slides, it is very likely that the structures in their arolia indeed produce and release secretions. A gland was also recently described for *Tipula* (Diptera; Friedemann et al., in press). Jiao et al. (2000) reported that the secretion in *Tettigonia viridissima* is transported through pore canals of the pad cuticle. However, the canals are only mentioned in the abstract and there are no images of them in the article.

The function of the gland is not entirely clear. The secretion probably plays a role in adhesion to smooth surfaces (e.g., Jiao et al., 2000; Orivel et al., 2001). However, in some species of Hymenoptera, the secretion is not released externally (Billen, 1986, Jarau et al., 2005), but remains within the pretarsus. A hydraulic function has been proposed by Federle et al. (2001). The liquid of the gland reservoir is pumped into the arolium, and this results in partial unfolding to increase the contact surface with the substrate. A hydraulic function seems unlikely in the case of Membracoidea, where the lobes of the arolium are not extendable. Even though oily footprints were visible on glass-slides in all examined members of Auchenorrhyncha, we could not find any canals or openings. However, this might also be due to fixation artifacts and the methods of examination. Presently, the mechanism of the release of glandular secretions remains unsolved. In *M. seminigra* (Hymenoptera, Apidae), secretion is produced by tendon glands and released at the base of the unguitractor plate into a slit between the last tarsal segment and the pretarsus (Jarau et al., 2004). The secretion contains pheromones which are used for communication in these social insects. However, as the glands we observed are not homologous with the tendon glands this mechanism can be excluded for Auchenorrhyncha with reasonable certainty.

In conclusion, we can say that the traditional definition of an arolium as a single hollow lobe between the claws (Dashman, 1953) is not always applicable. In fact, in most cases they are not hollow but filled with gland tissue. Moreover, arolia can be distinctly bilobed. Following the traditional definition, the two lobes of Membracoidea would be addressed as pulvilli. However, the comparison of the sclerotization pattern, the thickened cuticle, and the gland tissue clearly shows the homology with the arolium occurring in related groups. Pulvilli lack a thickened cuticle and gland tissue in their lumen and the equipment with sclerites (if present) is distinctly different (K. Friedemann, personal observation: several species of Pentatomomorpha). Therefore, we suggest an extended definition of arolium: a lobe between the claws, either hollow or filled with gland tissue; virtually always with a distinctly thickened cuticle consisting of chitinous rods in the distal part. The arolium can be distinctly bilobed but the equipment with sclerites is homologous to that of single-lobed arolia.

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3.3 Study III

Friedemann K, Schneeberg K, Beutel RG (2014)

Fly on the wall – attachment structures in lower Diptera

Systematic Entomology 30, 460-473

Abstract: Pretarsal attachment structures of representatives of the megadiverse Diptera were examined and documented, mainly using scanning electron microscopy. The focus is on the basal "nematoceran" lineages. The diversity is much higher than suggested by brief summarizing accounts in earlier studies. Both hairy and smooth attachment structures occur. A well-developed, pad-like empodium with its ventral surface covered with adhesive hairs is arguably a groundplan feature of Diptera. Very often this pad is combined with the presence of hairy pulvilli. However, smooth pulvilli occur in two of the examined groups. A smooth arolium is present in Tipulomorpha and likely an autapomorphy of this clade, i.e. secondarily acquired. Evolutionary transformations are interpreted based on recently published dipteran phylogenies.

Significance in the present thesis: This study focuses on the evolution of attachment devices in Diptera. Pretarsal structures of 32 species are described, illustrated and their evolutionary changes interpreted based on recently published dipteran phylogenies.

Own contribution: 70%

Systematic Entomology

Systematic Entomology (2014), **39**, 460–473

Fly on the wall – attachment structures in lower Diptera

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Abstract. Pretarsal attachment structures of representatives of the megadiverse Diptera are examined and documented, mainly using scanning electron microscopy. The focus is on the basal 'nematoceran' lineages. The diversity in structures is much higher than suggested by brief summarizing accounts in earlier studies. Both hairy and smooth attachment structures occur. A well-developed, pad-like empodium with its ventral surface covered with adhesive hairs is arguably a groundplan feature of Diptera. Very often this pad is combined with the presence of hairy pulvilli. However, smooth pulvilli occur in two of the examined groups. A smooth arolium is present in Tipulomorpha and likely an autapomorphy of this clade, suggesting that it was acquired secondarily. Evolutionary transformations are interpreted based on recently published dipteran phylogenies.

Introduction

Dipteran species are usually considered a nuisance or even as a serious threat as vectors of diseases (e.g. *Anopheles*, Tsetse fly). However, their exceptional flying abilities and their capacity to walk effortlessly on polished vertical surfaces (e.g. windows) or on ceilings have also fascinated observers including scientists (Bauchhenß & Renner, 1977). The attachment devices making this possible are the main topic of this contribution.

Diptera contain approximately 154 000 described species and are one of the extremely species-rich groups of Holometabola (Grimaldi & Engel, 2005). Their monophyly has never been questioned and is supported by convincing apomorphies such as the metathoracic halteres and the labial palpi transformed into labellae (Hennig, 1973). Adult dipterans are relatively homogenous in their morphology. By contrast the larvae are highly variable and the group has been very successful in colonizing a broad range of different habitats (e.g. Wiegmann *et al.*, 2011). Dipterans occur on all continents including Antarctica and in all zoogeographic regions.

The remarkable diversification (Wiegmann *et al.*, 2011) was accompanied by the evolution of different kinds of attachment structures. Functional principles (Niederegger & Gorb, 2003) and the morphology of attachment pads have been described for different members of Brachycera (e.g. Bauchhenß & Renner, 1977; Walker *et al.*, 1985; Gorb, 1998). However, surprisingly, the adhesive devices of lower Diptera ('Nematocera') have been greatly neglected so far. When characters of pretarsal structures have been included in several studies (e.g. Oosterbroek & Courtney, 1995; Sinclair & Cumming, 2006), usually they have not been illustrated.

Traditionally Diptera is divided into 'Nematocera' and Brachycera (e.g. Hennig, 1953). The former are a paraphyletic grade containing all nonbrachyceran groups (Hennig, 1973). The neutral term 'lower Diptera' is used in more recent contributions (e.g. Yeates *et al.*, 2007; Wiegmann *et al.*, 2011; Lambkin *et al.*, 2013). In contrast, the monophyly of Brachycera is well supported and undisputed (Hennig, 1973; Woodley, 1989; Yeates *et al.*, 2007).

The lower Diptera contain 33 families, 19 of which are extant (see Pape *et al.*, 2011). They were traditionally assigned to six groups: Culicomorpha, Ptychopteromorpha, Blephariceromorpha, Bibionomorpha, Psychodomorpha and Tipulomorpha (Oosterbroek & Courtney, 1995; Yeates *et al.*, 2007). A different classification containing ten extant groups is presented by Pape *et al.* (2011). The composition and phylogenetic relationships of these lineages is still controversial. Traditionally, Tipulomorpha (exclusive or inclusive of Trichoceridae) were considered as one of the 'most primitive' lineages of Diptera (Hennig, 1973; Wood & Borkent, 1989; Sinclair, 1992; Shcherbakov *et al.*, 1995; Michelsen, 1996; Beutel & Gorb, 2001; Blagoderov *et al.*, 2007; Stary, 2008). Other studies support the basal position of a clade comprising Culicomorpha and Ptychopteromorpha (Oosterbroek & Courtney, 1995; Yeates and Wiegmann, 1999, 2005; Yeates *et al.*, 2007). Recent analyses suggest a basal position

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for the highly specialized families Deuterophlebiidae (Bertone *et al.*, 2008; Wiegmann *et al.*, 2011) or Nymphomyiidae [Lambkin *et al.*, 2013 (Deuterophlebiidae were not included in the taxon sample)]. A basal position of Nymphomyiidae had been proposed previously by Hackmann & Väisänen (1982).

Both smooth and hairy attachment pads occur in Diptera (see glossary for definitions of pretarsal structures). An unpaired membranous lobe between the claws – an arolium – is present in Tipulomorpha, whereas a narrow or broadened empodium and paired hairy pulvilli occur in most lineages (e.g. Beutel & Gorb, 2001). However, detailed information on these structures in specific groups was scarce. Consequently, the primary aim of our study was to provide descriptions and illustrations of different kinds of dipteran adhesive devices, with the main focus on the 'nematoceran' lineages. We discuss the attachment structures with respect to functional aspects and the specific life habits of dipteran groups. Evolutionary scenarios for the different types of adhesive devices are developed based on recent phylogenetic studies, especially Wiegmann *et al.* (2011): a comprehensive contribution based on 30 kb from 14 nuclear loci, complete mitochondrial genomes and 371 morphological characters.

Material and methods

Examined taxa

We investigated 26 representatives covering most of the families of lower Diptera, six members of Brachycera and ten outgroup taxa. The nematoceran taxon sampling was largely adjusted to that of the Flytree-project (NSF: Assembling the Tree of Life, http://wwx.inhs.illinois.edu/research/flytree; see also Wiegmann *et al.*, 2011). The list of species used for the present study (including locality and fixation) is given in Table 1.

Scanning electron microscopy (SEM)

For scanning electron microscopy (Philips XL30 ESEM), specimens were completely dehydrated with ethanol (100%) over several stages and dried using HMDS (Hexamethyldisilazane; Brown, 1993), sputter-coated with gold (Emitech K500; Quorum Technologies, West Sussex, U.K.), and fixed on a rotatable specimen holder (Pohl, 2010). Scandium software (Soft Imaging System, Münster, Germany) was used for obtaining high-resolution images.

Cladistic analysis

In order to evaluate the character evolution of pretarsal structures we mapped the observed character states onto the cladogram of Wiegmann *et al.* (2011, Fig. 1) using the function implemented in Mesquite (trace characters over trees, reconstruction method parsimony ancestral states; Maddison & Maddison, 2011). Outgroups were arranged after Beutel *et al.* (2011) and Friedrich & Beutel (2010). Three tipuloid families (Pediciidae, Limoniidae and Cylindrotomidae) were included in our taxon sample but not in the study of Wiegmann *et al.* (2011). Their relationships were determined based on the study of Neugart *et al.* (2009).

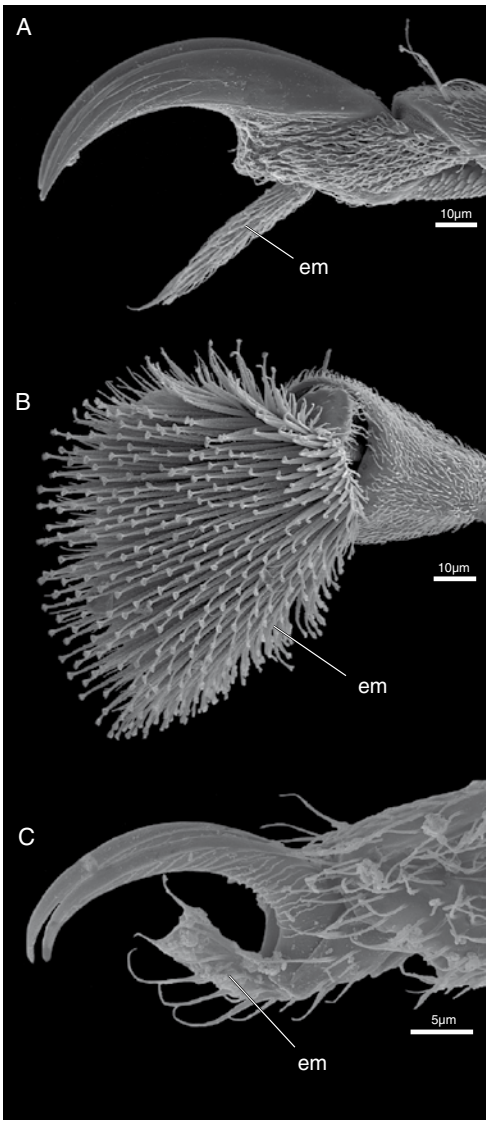

Fig. 1. Tarsi of *Deuterophlebia* and *Nymphomyia*, SEM images. (A) *Deuterophlebia coloradensis* female, lateral view; (B) *Deuterophlebia coloradensis* male, frontolateral view; (C) *Nymphomyia dolichopeza*, lateral view. Abbreviations: em, empodium.

Histology

Tarsi were embedded in Araldit CY 212s (Agar Scientific, Stansted/Essex, England) for sectioning. Cross-section (1-µm)

Table 1. List of Diptera taxa examined.

Taxon		Fixation	Locality
Diptera			
Anisopodidae	*Sylvicola fenestralis* (Scopoli, 1763)	70% EtOH	Germany, Thuringia
Asilidae	*Stilpnogaster aemula* (Meigen, 1920)	70% EtOH	Germany, Thuringia
Axymyiidae	*Axymyia furcata* McAtee, 1921	95% EtOH	USA, North Carolina
Bibionidae	*Bibio marci* Linnaeus, 1758	70% EtOH	Germany, Thuringia
Blephariceridae	*Edwardsina gracilis* Edwards, 1929	70% EtOH	Chile, Las Lagos
Cecidomyiidae	*Mayetiola destructor* (Say, 1817)	70% EtOH	In culture (Entomology Department, North Dakota State University, USA)
Ceratopogonidae	gen. sp.	70% EtOH	Germany, Thuringia
Chaoboridae	*Chaoborus christallinus* (De Geer, 1776)	70% EtOH	In culture (Institut für Spezielle Zoologie und Evolutionsbiologie, Germany)
Chironomidae	gen. sp.	70% EtOH	Germany, Thuringia, Jena
Corethrellidae,	*Corethrella appendiculata* Grabham, 1906	70% EtOH	In culture (Florida Medical Entomology Laboratory, USA)
Culicidae	*Anopheles maculipennis* (Meigen, 1818)	70% EtOH	Germany, Nordrhein-Westfalen
Cylindrotomidae	*Cylindrotoma distictissima* (Meigen, 1818)	70% EtOH	Italy, Piemonte, Cuneo Marora, San Sebastiano
Deuterophlebiidae	*Deuterophlebia coloradensis* Pennak, 1945	95% EtOH	USA, Colorado
Drosophilidae	*Drosophila melanogaster* Meigen, 1830	FAE	In culture (Wildtype Canton S, MPI for Chemical Ecology, Germany)
Glossinidae	*Glossina palpalis gambiensis* (Vanderplank, 1911)	FAE	In culture (International Atomic Energy Agency, Austria)
Limoniidae	*Antocha* sp. Osten Sacken, 1860	70% EtOH	Germany, Thuringia
Keroplatidae	*Macrocera centralis* Meigen, 1818	70% EtOH	Germany, Thuringia
Mycetophilidae	*Exechia separata* Lundstrom, 1921	70% EtOH	Germany, Thuringia
Nymphomyiidae	*Nymphomyia dolichopeza* Courtney, 1994	95% EtOH	USA, North Carolina
Pediciidae	*Pedicia rivosa* Linnaeus, 1758	70% EtOH	Italy, Piemonte, Torina
Psychodidae	*Psychoda alternata* Say, 1824	70% EtOH	Germany, Hamburg
Ptychopteridae	*Ptychoptera contaminata* Linnaeus, 1758	70% EtOH	Germany, Thuringia
Scatopsidae	*Coboldia fuscipes* (Meigen, 1830)	95% EtOH	USA, specific location unknown
Sciaridae	*Spathobdella falcifera* (Lengersdorf, 1933)	70% EtOH	Germany, Thuringia
Simuliidae	*Simulium* sp. Latreille, 1802	70% EtOH	Germany, Thuringia
Stratiomyiidae	*Pachygaster atra* (Panzer, 1798)	70% EtOH	Germany, Thuringia
Syrphidae	*Episyrphus balteatus* (De Geer, 1776)	FAE	Germany, Thuringia
Tabanidae	*Tabanus* sp. Linnaeus, 1758	70% EtOH	Germany, Thuringia
Tanyderidae	*Mischoderus* sp. Handlirsch, 1909	70% EtOH	New Zealand, Mount Arthur
Thaumaleidae	*Androprosopa* sp. Mik, 1898	95% EtOH	USA, Oregon
Tipulidae	*Tipula maxima* Poda, 1761	70% EtOH	Germany, Thuringia
Trichoceridae	*Trichocera fuscata* Meigen, 1818	70% EtOH	Germany, Thuringia
Outgroups			
Hymenoptera	*Xyela* sp.	70% EtOH	Germany, Thuringia
Neuroptera	*Chrysopa perla* (L., 1758)	70% EtOH	Germany, Thuringia
Lepidoptera	*Micropterix* sp.	70% EtOH	Germany, Thuringia
Strepsiptera	*Mengenilla chobauti* (Hofeneder, 1910)	–	Data taken from Pohl & Beutel (2004)
Mecoptera	*Nannochorista* sp.	70% EtOH	Australia, Tasmania
	Caurinus dectes Russell, 1979	70% EtOH	USA, Oregon
	Panorpa communis L., 1758	70% EtOH	Germany, Thuringia
	Merope tuber Newman, 1838	70% EtOH	USA, Iowa
	Boreus westwoodi Hagen, 1866	70% EtOH	Germany, Thuringia
	Hylobittacus apicalis (Hagen, 1861)	70% EtOH	USA, Illinois
Siphonaptera	*Ctenocephalus felis* (Bouché, 1835)	70% EtOH	Germany, Thuringia

Abbreviation: EtOH, ethanol; FAE, formaldehyde-ethanol-acetic acid (3:6:1).

series were carried out with a HM 360 (Microm, Walldorf, Germany) microtome. The sections were stained with Toluidin blue and Pyronin G (Waldeck GmbH and Co.KG/Division Chroma, Münster, Germany) and documented with AnalySIS software (Soft Imaging Systems, Münster, Germany).

Glossary

The terms used here are in accordance with the definitions of Dashman (1953), Richards & Richards (1979) and Beutel & Gorb (2001).

Acanthae: projections of the cuticle of unicellular composition with no sockets or sense cells.

Arcus: U-shaped band, which embraces the base of the arolium ventrally with its arms extending distally in the lateral walls on either side.

Arolium: the median lobe between the claws of the pretarsus. It can be completely membranous or partly sclerotized.

Auxiliae: lateral sclerites beneath the bases of the claws (= Basipulvilli s. Dashman, 1953).

Empodium: a median process between the pulvilli. It arises from the distal end of the unguitractor plate, is spine-shaped or lobe-like, and is often similar in shape to the pulvilli.

Manubrium: elongate median sclerite in the dorsobasal region of the arolium. It is articulated proximally on the end of the tarsus between the bases of the claws and by its narrowed distal end it is attached like a handle to the base of the arolium.

Mediolobus: pad-like empodium, possibly homologous to the arolium.

Pretarsus: the apical part of the hexapod leg, closely associated with the distal end of the terminal tarsomere.

Pulvilli: smooth or hairy paired lateral membranous lobes ventral to the claws. They are located on the auxiliae, which participate in control of pulvilli movements.

Unguitractor plate: median basal plate of the ventral surface of the pretarsus. It is usually invaginated into the end of the terminal tarsomere. Its surface is highly variable.

Very often the capitate acanthae of pulvilli or empodia of flies are referred to as 'tenent hairs' or 'tenent setae' (e.g. Niederegger *et al.*, 2002). However, the structures in the examined Diptera are not true setae, but acanthae (= single sclerotized protuberances originating from a single cell; see Richards & Richards, 1979). Therefore, in this study we refer to them as capitate or tenent acanthae.

Results

Nymphomyiidae (Nymphomyia), Deuterophlebiidae (Deuterophlebia) (Fig. 1)

A lobe-like empodium (em, Fig. 1C) bearing few long acanthae is present in *Nymphomyia* and the simple claws are well developed. The claws are strongly developed in females of *Deuterophlebia*. A well-developed, rather thick and cone-shaped empodium (em, Fig. 1A) is present and sparsely covered with acanthae. In males the empodium is modified to an extremely large lobe-like attachment pad covered with tenent acanthae (Fig. 1B).

Tipulomorpha (Figs 2–4)

Trichoceridae (*Trichocera*), Pediciidae (*Pedicia*), Limoniidae (*Antocha*), Cylindrotomidae (*Cylindrotoma*), Tipulidae (*Tipula*).

A smooth arolium (ar) is present in *Cylindrotoma* (Fig. 2D), *Tipula* (Fig. 2A), *Trichocera* (Fig. 2E, F) and *Pedicia* (Fig. 2B). It is small in the latter genus but well developed in the others. A distinct claw tooth is present in females of *Tipula paludosa*, but not in *Tipula maxima*. In *Antocha* (Fig. 2C) an arolium is absent, but an empodium (em) consisting of several microtrichiae is present. The claws bear four pronounced claw teeth (clt) and at the base of each of them a field with a group of long microtrichiae.

A cross-section of the pretarsus of *Tipula* shows the presence of a gland inside the arolium (agl, Fig. 3), although no specific gland openings could be found. The distal surface of the arolium is lamellate and the cuticle partly sclerotized. A long U-shaped sclerite with uncertain homology (see discussion) is present between the unguitractor plate (ut) and the base of the arolium. It arches upwards laterally (arc, Fig. 4B, C). Auxiliae, planta and manubrium are absent. One large sclerite is covering the dorsal side of the arolium except for its proximal part (dpl, Fig. 4A, C).

Culicomorpha (Fig. 5)

Corethrellidae (*Corethrella*), Chaoboridae (*Chaoberus*), Culicidae (*Anopheles*), Thaumaleidae (*Androprosopa*), Simuliidae (*Simulium*), Ceratopogonidae, Chironomidae.

A great variety of attachment devices occurs in Culicomorpha. In *Androprosopa* (Fig. 5A) an elongate empodium is present, with flat acanthae arising on both sides. A thin, long and smooth structure, possibly a modified pulvillus, arises below each claw (pu, Fig. 5A). An empodium of the same shape as in *Androprosopa* was found in a member of Ceratopogonidae and in *Anopheles* (Fig. 5C). However, in these cases pulvilli or other adhesive devices are lacking. Hairy, lobe-like pulvilli are present in *Chaoborus* (Fig. 5F) and the examined representative of Chironomidae (Fig. 5D). A row of acanthae likely representing an empodium is present in *Chaoborus*, whereas the empodium is spine-like in Chironomidae.

A reduced empodium is present in *Corethrella* and all tarsomeres are covered with long setae, which extend beyond the claws. Well-developed smooth pulvilli are present in *Simulium* (pu, Fig. 5B). Their dorsal surface is strongly striated and they arise from underneath the claws, but curve upwards so that their distal part is distal to the claws. The empodium is almost plate-like and sparsely covered with microtrichiae. Additionally, a hairy pad densely covered with small microtrichiae is present on the ventral side of the fourth tarsomere (Fig. 5E).

Psychodomorpha + Ptychoptera (Fig. 6)

Tanyderidae (*Mischoderus*), Ptychopteridae (*Ptychoptera*), Psychodidae (*Psychoda*), Blephariceridae (*Edwardsina*).

Attachment structures are absent in *Edwardsina*. Each claw bears a row of seven well-developed claw teeth. The distal tooth is the largest but proximally the size decreases and the teeth are closer. In *Ptychoptera* an empodium is present as a big hairy median lobe (Fig. 6A). The pulvilli are strongly reduced

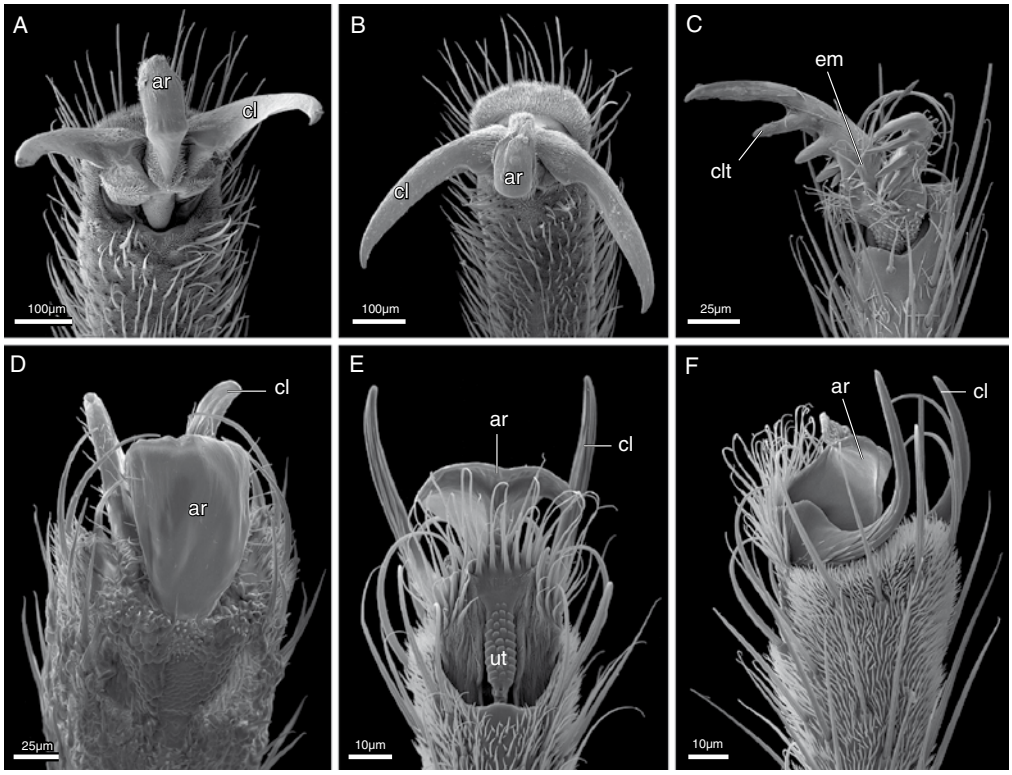

Fig. 2. Tarsi of Tipulumorpha, SEM images. (A) *Tipula maxima*, ventral view; (B) *Pedicia rivosa*, frontal view; (C) *Antocha* sp., ventrolateral view; (D) *Cylindrotoma distinctissima*, ventrolateral view; (E) *Trichocera fuscata*, ventral view; (F) *Trichocera fuscata*, lateral view. Abbreviations: ar, arolium; cl, claws; ctl, clawtooth; em, empodium; pu, pulvillus; ut, unguitractor plate.

in size and lack tenent acanthae. Attachment pads are absent in *Mischoderus* (Fig. 6B). The tarsus is completely covered with scale-like setae in *Psychoda* (Fig. 6C). A round, lobe-like empodium with tenent acanthae is present, whereas pulvilli are lacking.

Bibionomorpha (Fig. 7)

Axymyiidae (*Axymyia*), Bibionidae (*Bibio*), Keroplatidae (*Macrocera*), Cecidomyiidae (*Mayetiola*), Sciaridae (*Spathobdella*), Mycetophilidae (*Exechia*), Scatopsidae (*Coboldia*), Anisopodidae (*Sylvicola*).

In *Mayetiola* (Fig. 7A), *Sylvicola* (Fig. 7B), *Axymyia* (Fig. 7D) and *Bibio* (Fig. 7F) hairy pulvilli and lobe-like empodia are present. The pulvilli are distinctly smaller than the empodia in *Axymyia*, *Sylvicola* and *Mayetiola*. The pulvilli of *Mayetiola* lack tenent acanthae. In *Exechia* (Fig. 7E) an empodium comprising five long acanthae is present and each of the claws bears a long tooth. The empodium is reduced to a group of few tenent acanthae in *Spathobdella* (Fig. 7C). The density of hairs on the pulvilli is also reduced, but all of them have a broadened tip. The shape of the apical region of the tenent acanthae differs distinctly. The tip is round in those on the empodium of *Sylvicola* (Fig. 7G), whereas the acanthae of the pulvilli are flattened (Fig. 7H). In *Bibio marci*, the distal part of the tenent acanthae is broadened and leaf-like with an acuminate apex (Fig. 7I).

Orthorrhapha (Fig. 8)

Stratiomyidae (*Pachygaster*), Tabanidae (*Tabanus*), Asilidae (*Stilpnogaster*).

Paired hairy pulvilli and an empodium are present in all investigated members of Orthorrhapha. The empodium is spine-like in *Stilpnogaster* (Fig. 8G) and covered with small and short microtrichiae. Hairy adhesive soles are present on the ventral side of the tarsal segments (Fig. 8H). The empodia of *Pachygaster* (Fig. 8A) and *Tabanus* (Fig. 8C) are well-developed hairy lobes very similar in size and shape to the pulvilli.

Cyclorrhapha (Fig. 8)

Syrphidae (*Episyrphus*), Drosophilidae (*Drosophila*), Glossinidae (*Glossina*).

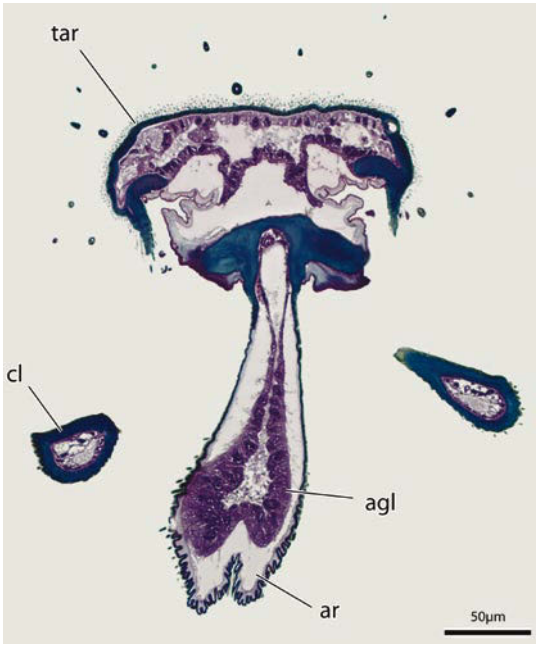

Fig. 3. Cross-section of pretarsus of *Tipula*. Abbreviations: agl, arolium gland; ar, arolium; cl, claw; tar, tarsus.

Well developed hairy pulvilli with their ventral surface covered with adhesive acanthae are present in *Episyrphus* (Fig. 8E) and *Glossina* (Fig. 8B, I, J). The empodium appears spine-like in both cases. In *Glossina* a field of microtrichiae is present proximad to the empodium. The empodium of *Drosophila* is divided into four thin spines (em, Fig. 8D). The pulvilli are cone-shaped and sparsely covered with a few capitate acanthae (pu, Fig. 8D). These acanthae are not restricted to the ventral side of the pulvilli, but present on the entire surface.

Character coding of tarsal structures:

1. Arolium: (0) absent, (1) present.
2. Pulvilli: (0) absent, (1) present.
3. Pulvilli: (0) present smooth, (1) present hairy.
4. Hairy pulvilli: (0) well developed as lobes, (1) small.
5. Hairy pulvilli: (0) capitate acanthae absent, (1) capitate acanthae present.
6. Hairy soles on tarsal segments: (0) absent, (1) present.
7. Claw teeth: (0) absent, (1) present.
8. Empodium: (0) absent, (1) present.
9. Shape of empodium: (0) present as a spine, (1) present as well developed lobe.
10. Vestiture of empodium: (0) densely covered with tenent acanthae, (1) number of acanthae reduced.

The different attachment structures are mapped onto the cladogram of Wiegmann *et al.* (2011: fig. 1) in Fig. 9.

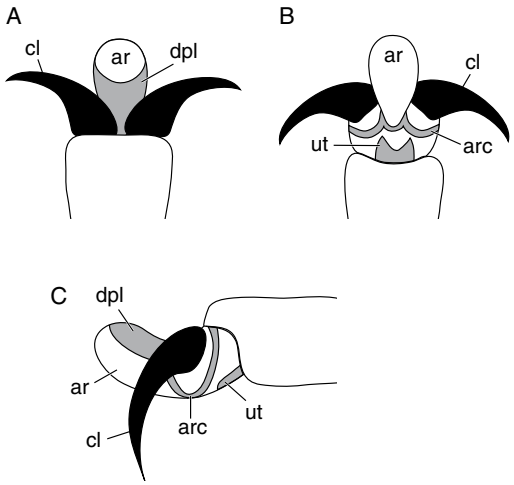

Fig. 4. Pretarsal sclerites of *Tipula*, sclerites grey, claws black. (A) dorsal view; (B) ventral view; (C) lateral view. Abbreviations: ar, arolium; arc, arcus; cl, claws; dpl, dorsal plate; ut, unguitractor plate.

Discussion

The following evolutionary interpretations are based on our structural observations, on earlier studies with a focus on functional aspects (e.g. Federle *et al.*, 2001; Gladun *et al.*, 2009), and on the comprehensive phylogenetic study by Wiegmann *et al.* (2011). Other phylogenetic concepts and their alternative implications for adhesive devices will be discussed briefly.

Diptera display a remarkable variety of attachment devices. This may be linked with their excellent flying abilities and the necessity to attach to and to move efficiently on and between a very broad variety of substrates, including numerous different plant surfaces, rotting plant and animal materials, faeces, and the integument of vertebrate hosts.

The principle attachment structures are the pretarsal arolium, paired pulvilli and the empodium, but tarsal attachment structures are also present in a few groups. Both hairy and smooth attachment devices occur (see Beutel & Gorb, 2001), but the former much more frequently. Hairy soles on tarsomeres formed by adhesive microtrichiae occur in two terminals of our taxon sample, *Simulium* (Culicomorpha, Fig. 5E) and *Stilpnogaster* (Brachycera, Fig. 8H). Apparently these are autapomorphic features of the respective taxa. The adhesive soles are structurally similar to those occurring in Strepsiptera (Pohl & Beutel, 2004: Stylopidia).

If the specific structure of the adhesive devices is taken into consideration (Figs 1, 2, 5–8), a remarkable diversity is revealed. Our results show a much higher variability than suggested by short summarizing accounts in earlier contributions, such as for instance Beutel & Gorb (2001), a study focused on interordinal relationships and the major evolutionary trends in the entire Hexapoda. All of the examined pretarsal (and tarsal) structures vary strongly in their shapes and configurations (or combinations thereof), even among relatively closely related

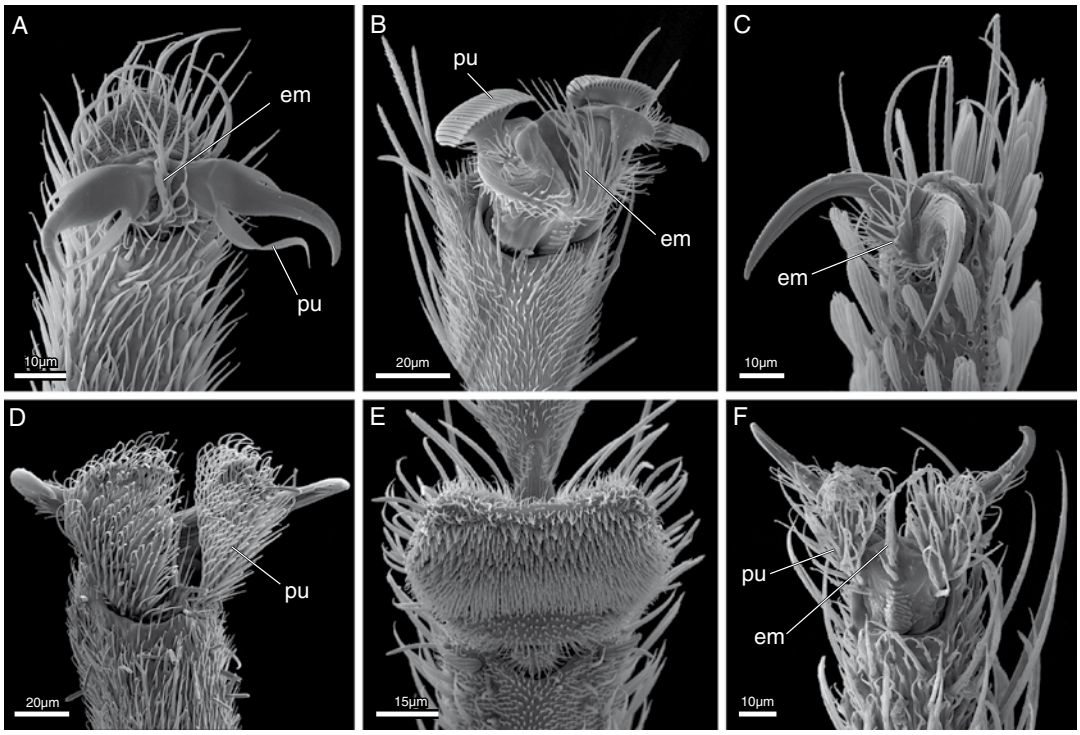

Fig. 5. Tarsi of Culicomorpha, SEM images. (A) *Androprosopa* sp., frontal view; (B) *Simulium* sp., ventrolateral view; (C) *Anopheles maculipennis*, ventrolateral view; (D) Chironomidae, ventral view; (E) *Simulium* sp., ventral view of hairy sole on fourth tarsal segment; (F) *Chaoborus chrystallinus*, ventral view. Abbreviations: em, empodium; pu, pulvillus.

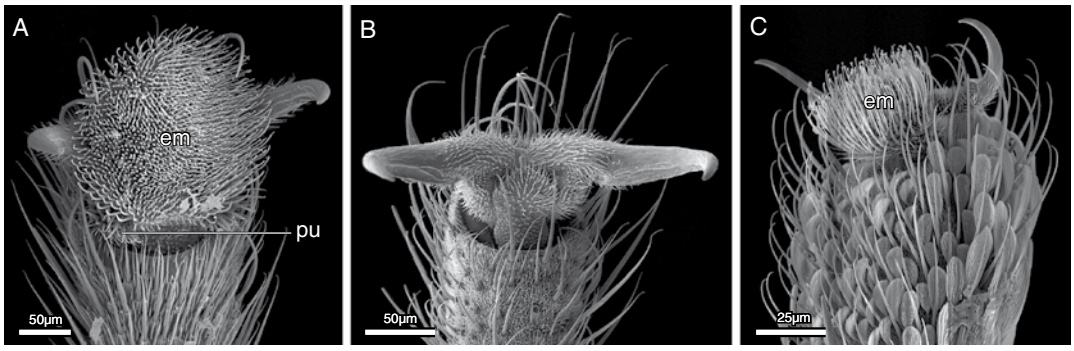

Fig. 6. Tarsi of Psychodomorpha. (A) *Ptychoptera contaminata*, ventral view; (B) *Mischoderus* sp., ventral view; (C) *Psychoda alternata*, ventrolateral view. Abbreviations: em, empodium; pu, pulvillus.

taxa. The tenent acanthae on different attachment devices also differ distinctly. Their tip is almost always distinctly broadened, but the shape varies. It can be round (e.g. *Sylvicola fenestralis*), triangular (e.g. *Stilpnogaster aemula*, *Glossina palpalis gambiensis*) or tapered (*Bibio marci*).

Of the examined species, only *Edwardsina* (Blephariceridae), *Mischoderus* (Tanyderidae) and *Antocha* (Limoniidae) completely lack attachment structures. Adult Blephariceridae rest by hanging from leaves or protruding stones with their forelegs (Courtney, 2000). For this behaviour claws are sufficient whereas specific attachment devices are not required. The lifestyle of Tanyderidae is largely unknown. The attachment devices of the presumably basal Nymphomyiidae and female Deuterophlebiidae are modestly developed at best, represented

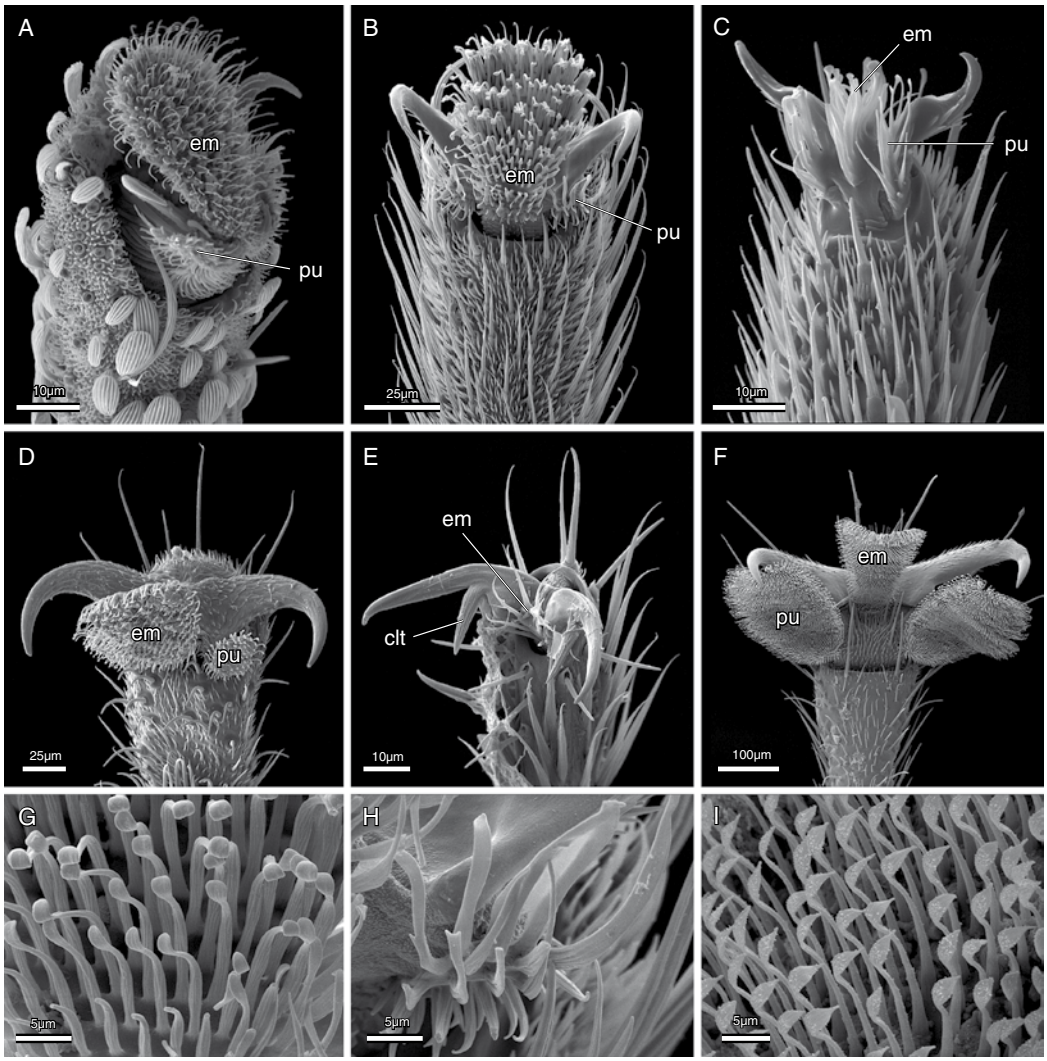

Fig. 7. Tarsi of Bibionomorpha, SEM images. (A) *Mayetiola destructor*, lateral view; (B) *Sylvicola fenestralis*, ventral view; (C) *Spathobdella falcifera*, ventral view; (D) *Axymyia furcata*, ventral view; (E) *Exechia separata*, frontal view; (F) *Bibio marci*, ventral view; (G) *Sylvicola fenestralis*, tenent microtrichia of empodium; (H) *Sylvicola fenestralis*, microtrichia of pulvillus; (I) *Bibio marci*, tenent microtrichia of pulvillus. Abbreviations: em, empodium; pu, pulvilli.

by a slightly modified empodium. In these cases the lack of elaborate adhesive pads may be linked with the short life-span and restricted activity of the adults, and in the case of Nymphomyiidae with extremely small size and partly submerged aquatic adult habits (Courtney, 1994). The large claws of Nymphomyiidae may provide better anchorage in the substrate of the streams where the adults copulate. The very large empodium of male deuterophlebiids, apparently linked with reduced claws, is very likely used during copulation in flight (Courtney, 1991), a specialized function different from the usual purpose of walking efficiently on different substrates (Beutel & Gorb, 2001). The males of *Deuterophlebia* are incapable of terrestrial locomotion (Courtney, 1991). They usually live close to streams, and the pad may also be used to break free from the stream surface and resume flight (Courtney, 1990).

Arolium

The answer to the crucial question of the groundplan condition of dipteran attachment structures remains ambiguous, especially with respect to the presence or absence of the most widespread

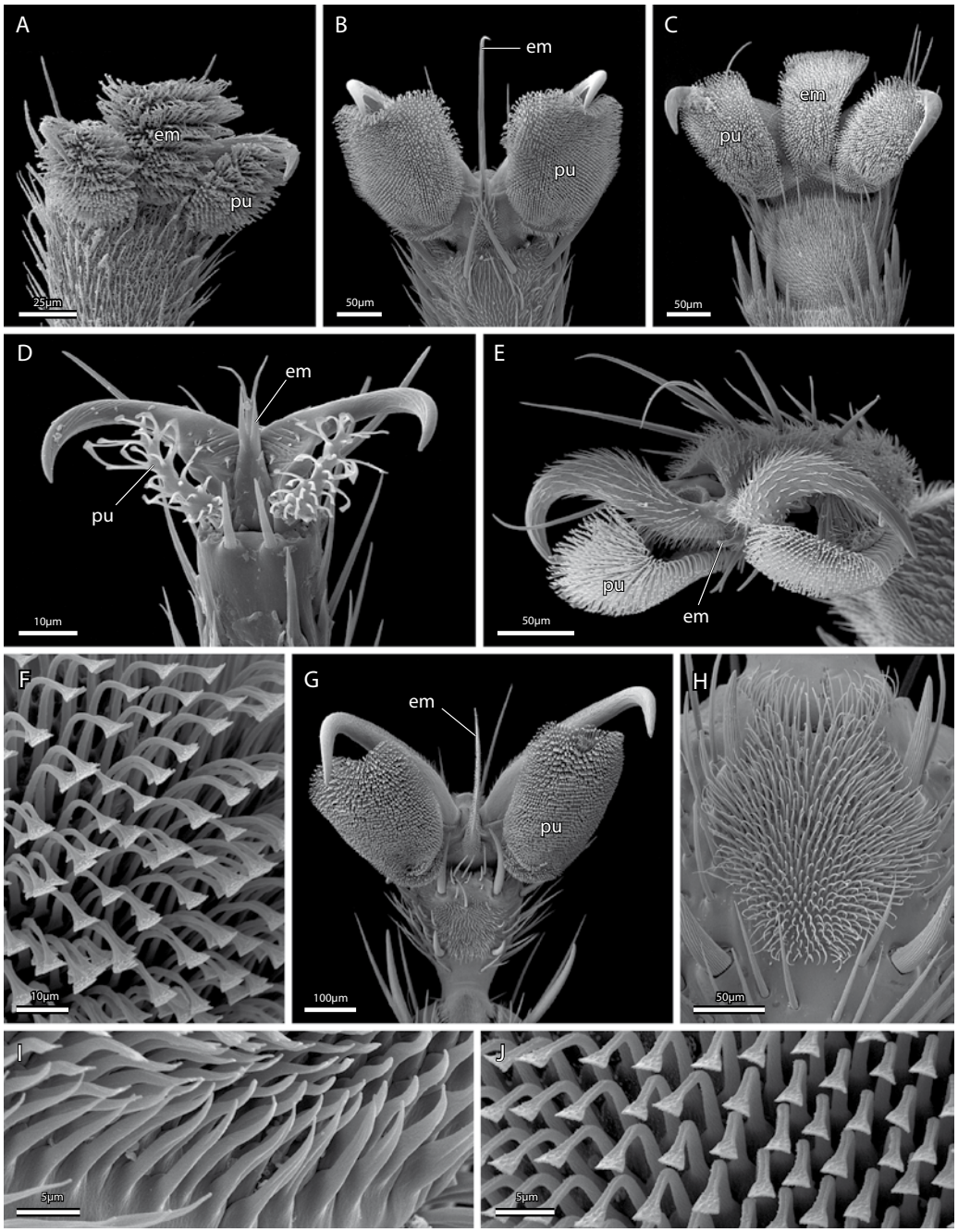

Fig. 8. Tarsi of Orthorrhapha and Cyclorrhapha, SEM images. (A) *Pachygaster atra*, ventral view; (B) *Glossina palpalis gambiensis*, ventral view; (C) *Tabanus* sp., ventral view; (D) *Drosophila melanogaster*, ventral view; (E) *Episyrphus balteatus*, frontal view; (F–H) *Silpnogaster aemula*; (F) tenent hairs of pulvillus; (G) *Silpnogaster aemula*, ventral view; (H) field of microtrichia on tarsal segment, ventral view; (I) *Glossina palpalis gambiensis*, tenent microtrichia proximal on pulvillus; (J) *Glossina palpalis gambiensis*, microtrichia distal on pulvillus. Abbreviations: em, empodium; pu, pulvillus.

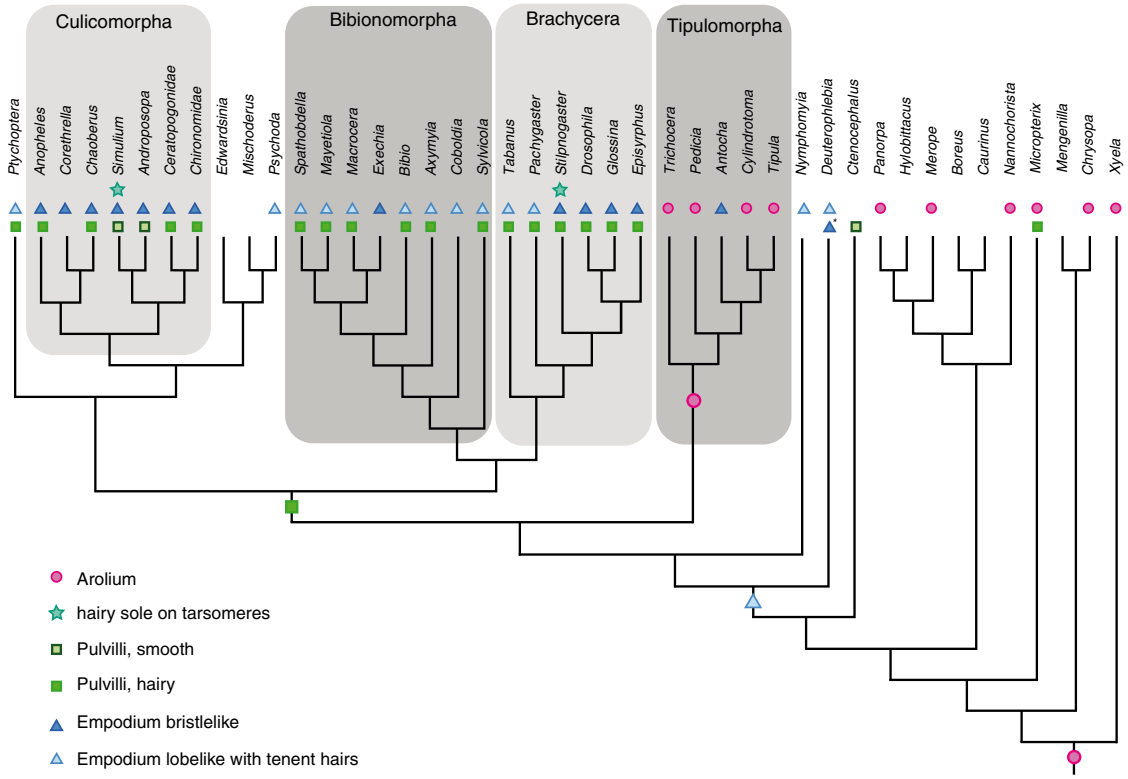

Fig. 9. Adhesive pad characters mapped on the cladogram of Wiegmann *et al.*, (2011: fig. 1). *The empodium is lobelike in males of *Deuterophlebia*, bristlelike in females.

insect attachment device – the arolium. This structure is commonly found in Neoptera (Pterygota excluding Odonata and Ephemeroptera) and is arguably a groundplan feature of this lineage (Beutel & Gorb, 2001, 2006; Friedemann *et al.*, 2014). Within Diptera it is only present in Tipulomorpha (with the exception of *Antocha*). The presence of the arolium was one of the arguments placing Tipulomorpha as the sister group of all other dipterans (e.g. Hennig, 1973). The evaluation of different morphological characters of adults and larvae yielded the same result (Wood and Borkent, 1989; Sinclair, 1992; Beutel & Gorb, 2001), and characters of the wing venation of extant and fossil taxa (Blagoderov *et al.*, 2007), and of the wing base (Stary, 2008), also supported this concept. However, recent studies suggest that Tipulomorpha are the sister group of Brachycera (Yeates & Wiegmann, 1999, 2005; Yeates *et al.*, 2007: both supertree-analyses) or nested within lower Diptera (e.g. Hackmann & Väisänen, 1982: morphological characters; Oosterbroek & Courtney, 1995: morphological characters of the larvae; Wiegmann *et al.*, 2011: molecular data; Lambkin *et al.*, 2013: morphological characters).

Two types of arolia occur in neopteran insects – those able to fold and spread, and those with a more or less constant shape. The arolium of Tipulomorpha belongs to the first type (Gladun *et al.*, 2009). Its sclerotization (Fig. 4) differs considerably from that in other holometabolan insects, such as Hymenoptera. Auxiliae and a planta or manubrium are absent. The U-shaped band that embraces the base of the arolium ventrally in Tipulomorpha resembles an arcus, which is the most vital element for unfolding the arolium. Ablation experiments have shown that spreading the arolium is not possible if this structure is removed (Frantsevich & Gorb, 2002).

The sclerite covering the dorsal surface of the arolium has been described before in *Tipula hortulana* (Gladun *et al.*, 2009). The authors refered to this element as dorsal plates, which act as extenders of the arolium. They maintain the narrow shape of the folded arolium like the cover spine of a book (Röder, 1986). On smooth surfaces, where claws do not find purchase, the claws diverge. The diverging claws flatten the extenders, thereby expanding the arolium and increasing the contact area between the attachment pad and the surface (Gladun *et al.*, 2009).

The presence of a gland inside the tipulomorph arolium (Fig. 3, agl) is an unusual feature and a potential autapomorphy. A so-called arolium gland occurs also in some Hymenoptera [Federle *et al.*, 2001: *Apis mellifera* (Apidae) and *Oecophylla smaragdina* (Formicidae); Jarau *et al.*, 2005: *Melipona seminigra* (Apidae)] but it is, in fact, located in the

proximal tarsal segment rather than in the arolium proper. In nonholometabolan insects a gland inside the arolium is only described for Mantophasmatodea (Eberhard *et al.*, 2009). However, it may be more common as it also occurs in both major lineages of Auchenorrhyncha (K. Friedemann, personal observation). The function of the gland remains unclear. Its secretion might play a role in adhesion to smooth surfaces (Orivel *et al.*, 2001); however, in some species the secretion is not released externally (*Polistes annularis* (Hymenoptera) Billen, 1986; *Melipona seminigra* (Hymenoptera) Jarau *et al.*, 2005) but remains instead within the pretarsus. The gland may also have a hydraulic function, as proposed by Federle *et al.* (2001). The liquid from the gland reservoir is pumped into the arolium, and this results in partial unfolding to increase the contact surface with the substrate.

Using the phylogeny of Wiegmann *et al.* (2011) (Fig. 9) the most parsimonious interpretation is that the arolium has evolved independently in Tipulomorpha, and is not homologous to the arolia in other holometabolan groups (e.g. Hymenoptera, Mecopterida, Lepidoptera and Neuroptera). Another possible interpretation would be the re-activation of a genetic program resulting in the secondary presence of this unpaired pretarsal attachment device. Controversial cases of suggested re-evolution of morphological structures have been discussed recently, including wings in advanced stick insects (Whiting *et al.*, 2003), digits in limbs of squamate reptiles (Brandley *et al.*, 2008), coiled shells in calyptraeids (Gastropoda; Collin & Cipriani, 2003), molar teeth in *Lynx* sp. (Kurtén, 1963), or mandibular teeth in frogs (Wiens, 2011). The most plausible explanation is that the master gene for the developmental pathway for the formation of a given structure evolved once, but the structure itself can appear several times through silencing and re-expression of the gene (Whiting *et al.*, 2003).

An entirely different interpretation would be the presence of the arolium in the dipteran groundplan (Beutel & Gorb, 2001), and secondary loss either once in a clade comprising Diptera excluding Tipulomorpha (e.g. Hennig, 1973; Wood & Borkent, 1989; Sinclair, 1992; Beutel & Gorb, 2001; Blagoderov *et al.*, 2007) or several times independently in different clades (e.g. Wiegmann *et al.*, 2011). This appears plausible as the loss of the arolium occurs frequently in insects (Beutel & Gorb, 2001, 2006) and there is no apparent reason why tipulomorph dipterans should have evolved (or re-activated) this structure. Moreover, the secondary absence in the potentially basal Nymphomyiidae and Deuterophlebiidae can be easily explained considering their specific biology (see above). This would leave another independent loss, as an apomorphy of Diptera excluding Nymphomyiidae, Deuterophlebiidae and Tipulomorpha (Fig. 9; Wiegmann *et al.*, 2011), linked with another derived feature supporting this lineage – newly acquired hairy pulvilli.

Pulvilli

Hairy pulvilli are present in Bibionomorpha, Culicomorpha and Brachycera. They are very likely secondarily missing in *Corethrella*, *Edwardsina*, *Mischoderus*, *Psychoda*, *Coboldia* and *Exechia*. The absence in Psychodomorpha is a potential apomorphy of this lineage.

The shapes of pulvilli differ considerably across the Diptera. In Brachycera the ventral surface of the pad-like pulvilli is covered with hundreds of capitate acanthae. The only exception within the examined Brachycera is *Drosophila*. The pulvilli of this genus are cone-shaped and sparsely covered with only few tenent acanthae that are not restricted to the ventral side of the pulvilli, but present across the entire surface. Pad-like pulvilli are also present in Chironomidae and *Chaoborus* (Culicomorpha), as well as in *Axymyia* and *Bibio* (Bibionomorpha). The pad is also present in *Axymyia* but distinctly reduced in size.

In some species the pulvilli are not pad-like, but merely a bundle of acanthae. This is the case in *Ptychoptera*, *Anopheles*, Ceratopogonidae, *Spathobdella*, *Mayetiola*, *Macrocera* and *Sylvicola*. Capitate acanthae are present in *Spathobdella*. With the exception of *Bibio* the pulvilli of Bibionomorpha are reduced in size and number of acanthae. The main attachment device in this lineage is a pad-like empodium.

Smooth pulvilli are present in *Simulium* and *Androprosopa*. They are similar to those occurring in fleas (Beutel & Gorb, 2001) but the phylogeny clearly suggests that they have evolved independently, as these taxa are deeply nested within lower dipteran lineages. The pulvilli of *Simulium* are well developed with a strongly striated dorsal surface. They arise from underneath the claws, but curve upwards so that their distal part is placed distad the claws. The pulvilli in *Androprosopa* arise from the same position, but are flattened like a banner and taper distally. They are probably not used for attachment to substrates. It is conceivable that they have a sensory function but evidence for this is presently lacking.

Pulvilli are widespread in hemimetabolan insects, but not as common in Holometabola. Apart from Diptera they occur only in Siphonaptera (smooth), Trichoptera (smooth) and some Lepidoptera (hairy). They usually function as attachment devices. However, in Lepidoptera their assumed function is to protect the arolium from abrasion on rough substrates (Al Bitar *et al.*, 2010).

Empodium

The presence of an empodium is a potential autapomorphy of Diptera. It is consistently missing in the other antliophoran groups and also in the nonantliophoran outgroup taxa. Its presence is very often linked with the presence of pulvilli. In our sampling both structures are present in 76% of the species. The shape of empodia is just as variable as the shape of the pulvilli.

A well-developed pad-like empodium with its ventral surface covered with tenent acanthae is arguably a groundplan feature of Diptera. However, this interpretation implies complete reduction in Tipulomorpha (excluding *Antocha*) (and secondary replacement with an arolium), and also secondary loss in *Mischoderus* and *Edwardsina*. The two genera *Tabanus* and *Pachygaster*, which are presumably close to the basal node of Braychycera,

are the only members of this lineage with well-developed, pad-like empodia and pulvilli. In the 'higher' Brachycera there is a trend towards reduction of the empodial pad. It is present as a long spine in the examined members of the 'higher' Brachycera (*Stilpnogaster*, *Episyrphus* and *Glossina*). A connection between the different types and the lifestyle of these taxa is not apparent. Tabanidae feed on the blood of warm-blooded vertebrates, whereas *Pachygaster* feeds on nectar and honeydew, just like most other members of Brachycera.

In all examined Culicomorpha and in *Spathobdella* and *Exechia* (Bibionomorpha) empodia are reduced in size and the number of acanthae are smaller and fewer. Within Culicomorpha the empodia of *Androprosopa* and Ceratopogonidae are very unusual, formed by a 'stem' with strands branching off on each side. This feathery empodium is probably not homologous with the superficially similar setiform empodium of higher Brachycera. The spine-like structure in Brachycera arises directly from the unguitractor plate (Röder, 1986), whereas the lobe-like empodium (often termed mediolobus) and its feathery equivalent arise from the membranous area distal to it. It is noteworthy that an arolium never occurs in combination with an empodium. A possible homology between the arolium and the hairy median lobe (mediolobus) of lower Diptera and lower Brachycera has been suggested, based on the origin of both from the area distad the unguitractor plate (Stuckenberg, 2001; Sinclair & Cumming, 2006). However, considering the completely different morphology of the ventral surface of the two structures this interpretation appears unlikely. Moreover, a largely or completely sclerotized arolium combined with a hairy surface (very short acanthae are present on the flexible arolium of Mantophasmatodea and basal Phasmatodea) does not occur in any other group of insects (Beutel & Gorb, 2001, 2006, 2008).

Conclusion

Tracing the precise evolutionary pathways of dipteran adhesive devices (e.g. arolium) is apparently a challenge, even though some phylogenetic interpretations appear straightforward, such as the presence of a distinctly developed empodium as a derived groundplan feature of Diptera, and hairy pad-like pulvilli as a groundplan autapomorphy of a clade including Psychodomorpha, Bibionomorpha, Culicomorpha and Brachycera.

What remains problematical is the connection between the general morphology (e.g. long and thin vs stout legs) and different life habits and preferred substrates, on the one hand, and the specific adhesive devices, on the other. There is no apparent configuration of attachment structures linked to either feeding on nectar or honeydew or sucking blood of vertebrates. Size reduction may have affected the complexity of attachment structures in some cases (e.g. Drosophilidae) but apparently not in others (e.g. *Mayetiola*, *Pachygaster*). In contrast to some specialized phytophagous insects in other groups, for instance beetles (see, e.g., Beutel & Gorb, 2001), dipterans seem to be less specific with respect to the substrates they attach to and walk on.

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3.4 Study IV

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Attachment forces of pea aphid host races on different legume species

Ecological Entomology

Abstract: This study deals with surface-related plant-insect interactions. The aphid species Acyrthosiphon pisum (pea aphid) encompasses genetically distinct sympatric host races. Each host race shows a preference for a certain legume species. The leaflet surfaces of these plants differ considerably in their wax coverage. Three host races were used to investigate whether the aphids showed differences in their attachment ability on the respective host and non-host plants. Aphid performance was tested by using traction force measurements. Surface morphology of plants and aphid tarsi was examined using SEM (scanning electron microscopy). The presence of wax blooms on the leaflets lowers the aphids' attachment ability considerably and diminishes their subsequent attachment on "neutral" surfaces like glass. The host races did not perform better on their host plants. All aphids, regardless of the original host plant, performed best on Vicia faba.

Significance in the present thesis: This study focuses on the interaction between plant surfaces and the attachment ability of aphids. Traction force measurements were carried out with 3 host-races of the pea aphid complex on four different plants. It was discussed if the ability to walk efficiently on plant surfaces plays a role on host fidelity and therefore speciation.

Own contribution: 60%

Attachment forces of pea aphid host races on different legume species

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Abstract

This study deals with surface-related plant-insect interactions. The aphid species *Acyrthosiphon pisum* (pea aphid) encompasses genetically distinct sympatric host races. Each host race shows a preference for a certain legume species. The leaflet surfaces of these plants differ considerably in their wax coverage. Three host races were used to investigate whether the aphids showed differences in their attachment ability on the respective host and non-host plants. Aphid performance was tested by using traction force measurements. Surface morphology of plants and aphid tarsi was examined using SEM (scanning electron microscopy). The presence of wax blooms on the leaflets lowers the aphids' attachment ability considerably and diminishes their subsequent attachment on "neutral" surfaces like glass. The host races did not perform better on their host plants. All aphids, regardless of the original host plant, performed best on *Vicia faba*.

Introduction

Aphids, among other phytophagous insects, are considered prime candidates for sympatric speciation related to their highly specialized association with their host plants (Via, 2001). They do not only feed on the plant but it serves also as their micohabitat and mating site. This close relationship leads to assorted mating and reduces the gene flow between the populations on different hosts. This in turn leads to the formation of varieties (commonly referred to as host races), which differ genetically from each other. Thus, host plant specialization may lead to complete speciation (Peccoud and Simon, 2010).

The pea aphid complex (*Acyrthosiphon pisum*) comprises at least 11 distinct host races living on different legume species (Peccoud et al., 2009). These varieties show differences in e.g. their reproductive mode (Frantz et al. 2006b), prevalence of facultative symbionts (Frantz et al., 2009b), defensive behavior (Kunert et al., 2010) or susceptibility to fungal pathogens (Ferrari & Godfray, 2003). Host races can be considered as an intermediate stage of speciation (Peccoud and Simon, 2010).

Studies have shown that host races usually show a higher mortality on non-host plants (Sandstrom and Pettersson, 1994; Schwartzkopf et al., under review), with the exception of *Vicia faba*. The fava bean can be considered as a universal host plant for *Acyrthosiphon pisum*.

There are many plant factors that play a role in host selection and ultimately aphid speciation. Chemical factors inside the plant tissue (sieve-elements, epidermis and mesophyll) seem to be important for host plant recognition and feeding behavior (e.g., Schwartzkopf et al., under review; Alvarez et al. 2006). Aside from these factors, the ability to walk efficiently on plant surfaces may also play a role in host fidelity and specialization.

Most plant surfaces are covered with trichomes or a waxy layer, which makes attachment and locomotion difficult for most insects (e.g., Barthlott 1998; Gorb & Beutel 2001). The presence of deterrent epicuticular lipids (Powell et al., 1999) and glandular trichomes (Alvarez et al., 2006) has been shown to affect aphid host selection. However, insects have evolved a variety of different attachment structures to cope with smooth and often slippery surfaces, on which claws alone find no purchase (for an overview on attachment structures in insects see e.g., Beutel & Gorb, 2001; Friedemann et al., in press). In oak-feeding aphids, the ability to attach successfully to the plant surface has likely contributed to the specialization on the specific plant substratum (Kennedy, 1986). The locomotion of the aphid *Tuberculoides annulatus*, which feeds on *Quercus robur*, is impeded on leaves of *Quercus ilex*. In contrast the aphid *Myzocallis schreiberi*, a specialist feeding on *Quercus ilex*, performs well on its host. It is conceivable that a similar specialization related to attachment and locomotion could be found in the pea aphid complex.

The aim of this study is to find out if there are any differences in the attachment capacity of three host-races of the pea aphid complex on host and non-host plants. We examined the attachment performance by measuring the traction forces on their respective host plants, the universal host *Vicia faba*, and on two non-host plants. Ultimately the goal is to find out whether the attachment capacity could be another factor contributing to the maintenance of the varieties. Additionally we tested whether walking on the different plants affects the ability to attach and walk on neutral surfaces.

Material and techniques

Scanning electron microscopy (SEM)

SEM-images were taken with a Philips XL30 ESEM (Fei, Eindhoven, the Netherlands). The plants were air-dried. The insects were completely dehydrated with ethanol (100%) over several stages, dried at the critical point (Emitech K850; Emitech, Ashford, Kent, UK) or treated with HMDS (hexamethyldisilazane; Brown, 1993), sputter-coated with gold (Emitech K500; Emitech), and fixed on a rotatable specimen holder (Pohl, 2010). Scandium software (Soft Imaging System, Münster, Germany) was used to obtain high resolution images.

Plants and insects

Four different legume species were used: *Medicago sativa* cv. "Giulia" (Appels Wilde Samen GmbH, Darmstadt, Germany), *Pisum sativum* cv. "Baccara" (S.A.S. Florimond Desprez, Cappelle-en-Pévèle, France), *Trifolium pratense* cv. "Dajana" (Appels Wilde Samen GmbH, Darmstadt, Germany), and *Vicia faba* cv. "The Sutton" (Nickerson-Zwaan, Made, The Netherlands). All plants were reared in pots of 10 cm diameter on plant substrate "Klasmann Tonsubstrat" (Klasmann-Deilmann GmbH, Geeste, Germany) in a climate chamber (20 °C, 70 % relative humidity, 16 hours light per day).

Three different *Acyrthosiphon pisum* clones occurring sympatrically in Western Europe were used. The aphid clones were collected in the field from three legume species: clone "L1_22" from *M. sativa*; clones "P136" from *P. sativum*; clone "T3_8V1" from *T. pratense* (for detailed clone information see Table S1 in Peccoud et al. (2009b). All aphid clones used in the experiment were maintained on their host plant covered with air-permeable cellophane

bags (Armin Zeller, Nachf. Schütz & Co, Langenthal, Switzerland) to prevent aphid cross-contamination. Conditions for all aphid rearing in this study were: 20 °C, 70 % relative humidity, 16 hours light per day.

Traction experiments with insects

Traction experiments with tethered walking aphids were carried out to measure insect attachment forces on different surfaces. Force tests were performed with a load cell force transducer (10 g capacity, Biopac Systems Ltd., Santa Barbara, CA, USA). Only adult wingless females were used in experiments. The aphids were attached to the force sensor with a hair (10–15 cm long) glued to the dorsal surface of the insect with a droplet of water soluble glue. Leaflets were cut off the plant and attached with double-sided adhesive tape to a horizontal glass plate. Five types of substrates were tested: (1) abaxial leaflet surface of *Pisum sativum*, (2) abaxial leaflex surface of *Vicia faba*, (3) abaxial leaflet surface of *Trifolium pratense*, (4) abaxial leaflet surface of *Medicago sativa* and (5) a glass plate as a control. Nine force tests were carried out with each individual aphid: first on the glass substrate, then on one of the plant surfaces, again on the glass surface, on another plant surface, again on the glass and so on. The force generated by the insect walking horizontally on test substrates was measured. Force–time curves, where the aphid stretched the hair for ca. 5-10 s, were used to estimate the maximal traction force of these insects. Tests were carried out at room temperature of 24–25 °C and 40–45% of relative humidity. For each plant surface type, experiments with 16 individual insects were conducted. In all, 48 insects were tested and 432 force measurements were performed. Statistic analyses were carried out with SigmaPlot 11.0.

Results

Morphology of aphid attachment structures

The tarsus of the pea aphid comprises two segments. The distal tarsomere is about three times longer than the proximal one. An eversible, membranous and cushion-like attachment pad (tp, Fig. 1) is present on the distal ventral part of the tibia. Additionally, setiform parempodia are inserted on the pretarsus. There are no apparent differences between the attachment pads of the examined races.

Leaflet surface morphology

There are distinct differences in the crystalline epicuticular wax coverage between the examined leaflet surfaces. The abaxial leaflet surface *in Trifolium pratense* (Fig. 2A) and *Medicago sativa* (Fig. 2B) is covered by small, flat wax platelets vertically oriented to the surface. They are about 0.7 µm long. The wax crystals in both plant species are almost identical in structure, size and density. The crystals on *Pisum sativum* (Fig. 2D) are very long (approx. 2.8 µm), flat and have fringed external edges. In most cases the orientation is perpendicular to the surface. In *Vicia faba* (Fig. 2C) crystalline waxes are missing on the leaf surface.

Traction forces of aphids on different leaflet surfaces

The maximal traction force generated on glass by the different aphid individuals varies significantly (0,17 - 0,87 mN). Therefore, we used data normalized to values obtained on

glass for comparison of different surfaces. For each individual the force obtained on a test plant surface was compared to that on glass (considered as 100%).

In all examined host-races the values of the maximal traction forces on *Medicago* and *Pisum* were significantly lower than those produced on the glass plate (Fig. 3A, Table 1, Kruskal-Wallis One Way Analysis of Variance on Ranks; *Pisum* race: H = 56.161 with 4 degrees of freedom (P = <0.001); *Trifolium* race: H = 59.625 with 4 degrees of freedom (P = <0.001); *Medicago* race: H = 55.914 with 4 degrees of freedom (P = <0.001)).

The mean traction forces in all examined aphid races were considerably reduced to only 2.89% (± 10.12%) on the pea and 16.96% (± 24.87%) on *Medicago* (compared to walking on glass). Walking on the pea was almost impossible for the insects, since they constantly slipped and basically walked on the spot. The insects performed well on *Vicia* and *Trifolium*, where they achieved mean maximum traction forces of 94.51% (± 37.45%) and 84.69% (± 43.71%), respectively.

Influence of walking on plants

Immediately after walking over the plant surfaces aphids were put on glass to test whether walking on the plant did have any lasting effect on the subsequent attachment ability on a "neutral" surface where they performed without problems earlier.

Walking on *Pisum* and *Medicago* did have a negative influence on the subsequent attachment ability of the aphids (Fig. 3B, Table 2). However, there are distinct differences between the races. The influence was not significant in the *Medigaco*-race. Compared to the attachment ability on glass before walking on a plant surface (100%) it was reduced to 80.41% (± 21.72%) in the *Pisum*-race, and 90.33% (± 55.16%) in the *Trifolium*-race after

161 walking on pisum leaflets. Walking over alfalfa leaflets significantly reduced the subsequent
162 attachment ability on glass to 80.29% (± 56.04%) in the *Trifolium*-race. Walking on *Vicia*
163 *faba* did not have any influence on the attachment ability afterwards.

164 For the force-time measurements we let each individual aphid pull on the hair three times.
165 For the subsequent calculations the mean force of the three sets was used. However, on
166 closer examination, the force-time curves show an interesting development after walking
167 over the leaflets of *Trifolium*, *Medicago* and *Pisum* (Fig. 4). In almost all examined
168 individuals we could observe the same pattern: the first time they pulled on the hair
169 (immediately after they were taken from the respective leaflets) the force was very low and
170 they lost their grip on the glass plate quickly. By the time of the third set the attachment
171 ability was restored and the aphids pulled with the same force as before they walked over
172 the plant surfaces. If we would only consider the force-time curves of the first of the three
173 sets, there would be a significant reduction of the attachment ability in all examined host
174 races after walking on *Pisum*, *Trifolium* and *Medicago*.

175

176 <u>Differences between the host races</u>

177 We tested whether the ability to walk on a certain plant surface differs between the races.
178 The performances of the different races on the same plant were compared. There were no
179 significant differences between the performance of the three aphid races on pea, bean and
180 clover. Only on alfalfa did we find a difference in the performance of the races ($H = 7.427$
181 with 2 degrees of freedom ($P = 0.024$). The aphids of the *Pisum*-race performed significantly
182 better than the other two varieties.

Discussion

The presence of wax blooms provides a defense against insect herbivores by impeding their attachment for feeding or oviposition (e.g. Brennan et al., 2001, Bodnaryk, 1992, White & Eigenbrode, 2000). The very low traction forces of aphids on pea leaflets can be explained by the structure of the wax platelets. The substrate probably contaminates the attachment structures, thus lowering the attachment ability of the insects distinctly (Rutledge & Eigenbrode, 2003; Gorb et al., 2008). Additionally, the presence of wax blooms increases the microscopic surface roughness, which in itself is sufficient to reduce insect attachment (Scholz et al., 2010). Scholz et al. (2010) showed that surface roughness within scales of a specific length prevents adhesion by creating a pattern too rough for adhesive pads but not rough enough for claws.

On *Vicia faba*, however, the attachment forces were even slightly higher than on the glass plate. On this plant no wax platelets are present that might have an influence on the attachment ability. The leaflets showed the highest density of stomata among the examined plants. These stomata increase the roughness of the plant surface, and probably provide better purchase for the insects.

Even though there was virtually no recognizable difference between the structure of the wax on the leaflet surfaces of *Trifolium* and *Medicago*, the performance of the aphids on these plants differed considerably. Attachment forces were much higher on *Trifolium* than on *Medicago*. This is apparently due to the presence of trichomes on the leaflet surface of *Trifolium*. During the experiments we observed the insects trying to hold onto the trichomes with their claws thus creating leverage to pull themselves forward. However,

205 they slipped whenever the apical part of their legs came into contact with the wax covered
206 surface. This also accounts for the high standard deviation observed on *Trifolium* (e.g. 48%
207 in the *Pisum* race (mean 0.33 mN ± 0.11)). Some individuals were stalking on top of the
208 trichomes relatively efficiently, while others tried to walk on the waxy surface and slipped.

209 The presence of wax platelets on the surfaces of the leaflets of the different plants also
210 explains the differences in the subsequent performance on glass. Aphids put onto a glass
211 plate immediately after walking on the respective leaflets had problems where they
212 performed well before. This reduced attachment ability is very likely due to contamination
213 of the attachment pads. After walking on *Vicia*, the only examined plant with no wax
214 platelets, the subsequent attachment ability was not affected.

215 This contamination effect does not last for long. After walking a couple of centimeters the
216 aphids were able to produce the previous traction forces. This suggests that they are able to
217 get rid of the waxes somehow. Grooming behavior is only known in some groups of insects
218 in this functional context, i.e. cleaning of the tarsi and pretarsal elements (e.g. Hlavac, 1975).
219 It has been shown that adhesive pads are able to "self-clean" with repeated steps (Clemente
220 et al., 2010). With each step they leave parts of the particles on the surface. In stick insects
221 the pads recovered 53.4% of the lost shear force after only eight steps (Clemente et al.,
222 2010). A similar procedure is conceivable in aphids, since the attained traction forces get
223 stronger with each step, even though the aphids don't show any active grooming behavior.

224 The aphid varieties did not perform better on their hosts compared to non-host plants. The
225 races only differed in their performance on alfalfa, where the *Pisum* race could walk
226 significantly better than the others. This suggests that the aphids are not better adapted to
227 walking on their host plants, and that chemical plant factors within the plant tissues (e.g.

228 Schwartzkopf et al., under review) are more important in the context of host plant choice
229 than the plant surface. It also has to be taken into consideration that aphids do not only feed
230 on the leaflets. They are often found on the stems and tendrils where attachment is easier.

231 Even though our results suggest that the attachment ability on plant surfaces does not play
232 a role in maintaining the host races, further research on this issue is necessary. As the wax
233 blooms can also reduce the attachment capacity of parasitoids and predators of aphids, it
234 can influence the regulation of aphid populations by their natural enemies. Populations of
235 predaceous coccinellids for instance did not differ consistently between reduced-waxbloom versus
236 normal-waxbloom peas, failing to support a hypothesis that predator populations are more dense
237 on reduced waxbloom peas (White & Eigenbrode, 2000). Therefore, further research on the
238 attachment ability of typical predators of pea aphids (e.g. hover flies, lady bugs) is necessary.

239

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243

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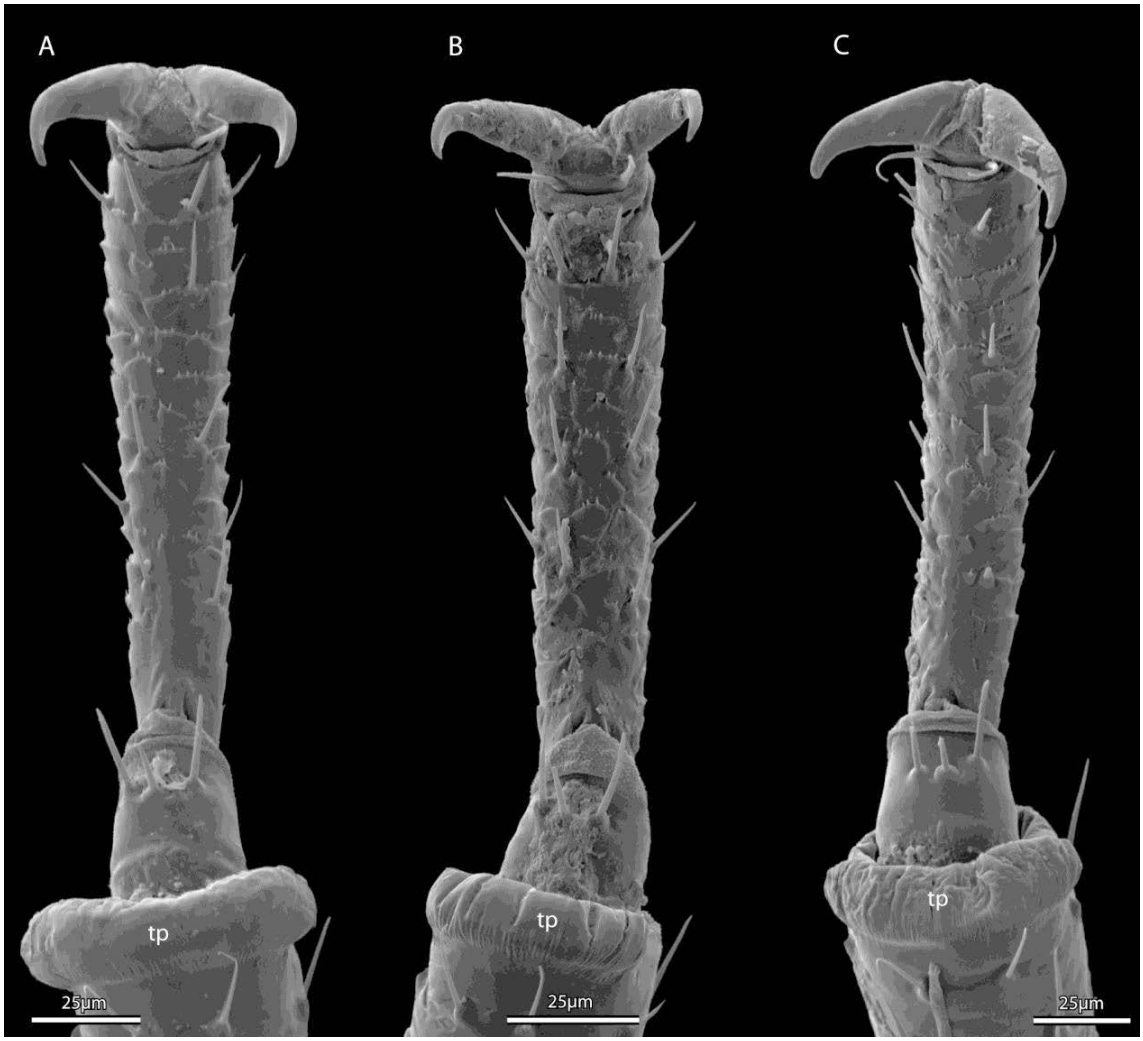

Fig. 1: SEM micrographs of the tarsi of three different host races *Acyrthosiphon pisum*. (A) *Trifolium* race (clone T3_8V1), (B) *Medicago* race (clone L1_22), (C) *Pisum* race (clone P136).

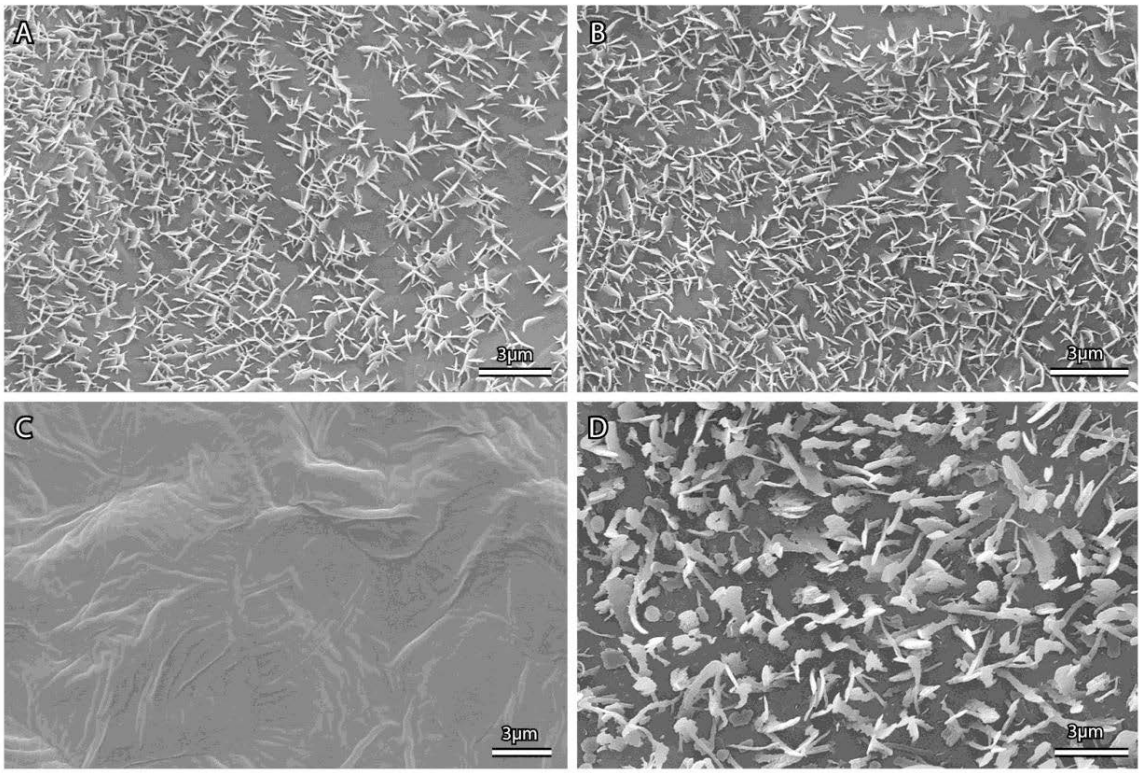

Fig. 2: SEM micrographs of the epicuticular wax coverage on the abaxial leaflet surfaces. (A) *Trifolium pratense*, (B) *Medicago sativa*, (C) *Vicia faba*, (D) *Pisum sativum*.

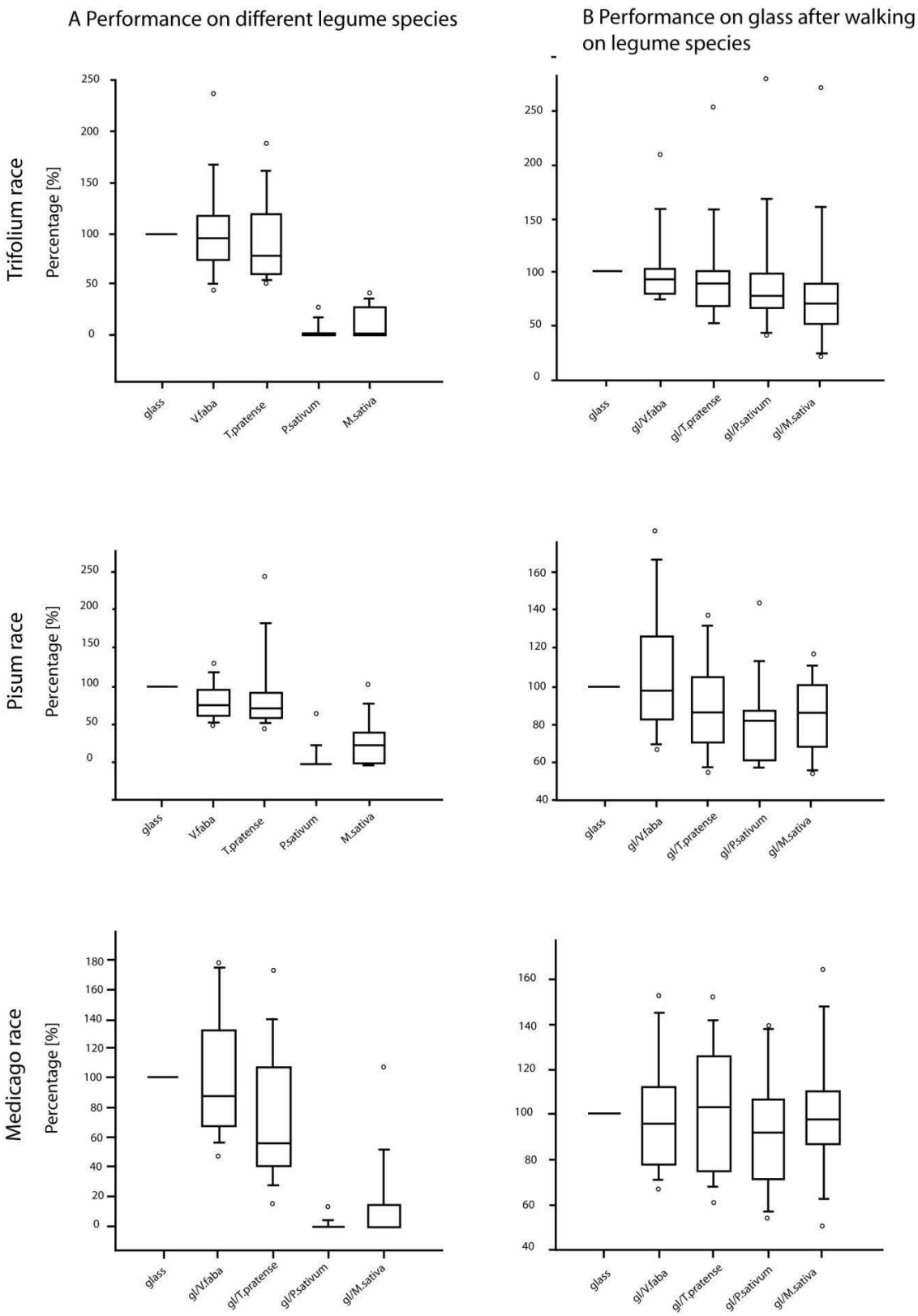

Fig. 3: Traction forces of aphids walking on different legume species and glass. Y - axis: traction force in percent, X - axis: substrates. (A) Performance on glass (100%) and on four legume species (*Vicia faba*, *Trifolium pratense*, *Pisum sativum*, *Medicago sativa*). (B) Performance on glass after the aphids were taken from the respective legume species (gl/V.faba = glass after walking on *Vicia faba* and so on).

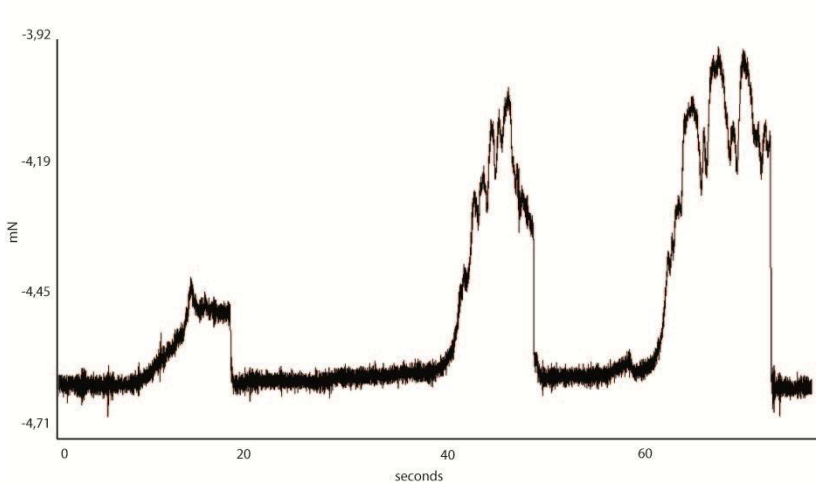

Fig. 4: Exemplary graph of the measured traction forces on glass of one aphid individual (*Medicago* race) immediately after it was taken from Medicago sativa. The three peaks in the curve show the three times the aphid pulled on a hair connected to the force sensor. X - axis: time in seconds, Y - axis: force in Millinewton.

4 Discussion

4.1 Evolution of attachment structures

Despite a very broad spectrum of structural variations, there are only two basic designs of attachment pads on insect legs: hairy and smooth (Beutel & Gorb, 2001).

Interestingly, hairy structures that occur in different lineages of Polyneoptera and Holometabola (Beutel & Gorb, 2001; 2006) are lacking on the tarsus and pretarsus of all acercarian subgroups. The location of different attachment devices varies considerably. They occur on the pretarsus as an unpaired pad-like arolium, as paired pulvilli, or as an unpaired sclerotized empodium; on the tarsus as smooth euplantulae; and even on the distal tibia as a tibial pad. In some aphids an inflatable attachment pad is present on the abdomen to help the insects turn over in case they land on their backs (Carver & White, 1971). While smooth attachment pads predominate in Acercaria, the opposite is true for Diptera. In this lineage hairy and smooth pulvilli, smooth arolia, hairy empodia of different shapes and sizes, and hairy tarsal soles occur.

Only very few of the examined insect species completely lack adhesive devices. In some cases they are reduced in size and very likely function as a sensory device. The lack of distinctly developed adhesive pads may be linked with a very short life span (e.g., females of Deuterophlebiidae, Diptera), miniaturization (Nymphomyiidae, Diptera), or a specific lifestyle not requiring attachment structures (e.g., ground-dwelling groups like Zoraptera, Leptodomorpha or Enicocephalomorpha (Heteroptera)). Surprisingly attachment pads are also missing in the ectoparasitic elephant lice. Apparently their main anchoring mechanism is the proboscis, which is usually firmly embedded into the skin of the host (Weber, 1969).

4.1.1 Evolutionary scenarios for attachment structures

A robust phylogenetic background is necessary for the reconstruction of evolutionary scenarios. Due to the ambiguities in the phylogenetic reconstruction of Acercaria (as well as in Diptera), the following considerations should be treated with some reservation. The results of our analysis (Study I) were compared to the latest results of the 1KITE project (unpublished, pers. comm. B. Misof), which suggest that Acercaria are paraphyletic, with Psocodea as the sistergroup of Holometabola. However, mapping the characters on the 1KITE phylogeny (analyses of transcriptomes of ca. 1000 species representing all insect orders) did not yield evolutionary scenarios differing from those suggested by our own analysis. Whether Acercaria are considered as monophyletic or not, the most parsimonious assumption is that arolia were reduced 4 times and that pulvilli evolved three times independently in the groups traditionally assigned to this lineage.

4.1.2 Arolium

The traditional definition of an arolium as a single hollow lobe between the claws (Dashman, 1953) is not always applicable. In fact, in most cases it is not hollow but filled with gland tissue. Moreover, arolia can be distinctly bilobed. Following the traditional definition the two lobes of Membracoidea would be addressed as pulvilli. However, the comparison of the sclerotization pattern, the thickened cuticle, and the gland tissue clearly shows that it is homologous with the arolium occurring in related groups. Pulvilli generally lack a thickened cuticle and gland tissue in their lumen, and the equipment with sclerites (if present at all) is distinctly different.

The arolium is likely a groundplan feature of Neoptera (Beutel & Gorb, 2001). Within Diptera it is only present in Tipulomorpha (with the exception of *Antocha*). Its preservation was one of the arguments placing Tipulomorpha as the sister group of all other dipteran lineages (e.g., Hennig, 1973). Using the phylogeny of Wiegmann et al. (2011) the most parsimonious interpretation is that the arolium has evolved independently in Tipulomorpha, and is not homologous to the arolia in other holometabolan groups (e.g., Hymenoptera, Mecopterida, Lepidoptera and Neuroptera). Another possible interpretation would be the re-activation of a genetic program resulting in the secondary presence of this unpaired pretarsal attachment device. Controversial cases of suggested re-evolution of morphological structures have been discussed recently, including wings in advanced stick insects (Whiting et al., 2003), digits in limbs of squamate reptiles (Brandley et al., 2008), coiled shells in calyptraeids (Gastropoda; Collin & Cipriani, 2003), molar teeth in *Lynx* sp. (Kurtén, 1963), or mandibular teeth in frogs (Wiens, 2011). The most plausible explanation is that the master gene for the developmental pathway for the formation of a given structure evolved once, but the structure itself can appear several times through silencing and re-expression of the gene (for summary see Whiting et al., 2003). An entirely different interpretation would be the presence of the arolium in the dipteran groundplan (Beutel & Gorb, 2001), and secondary loss either once in a clade comprising Diptera excluding Tipulomorpha (e.g., Hennig, 1973; Wood & Borkent, 1989; Sinclair, 1992; Beutel & Gorb, 2001; Blagoderov et al., 2007) or several times independently (e.g., Wiegmann et al., 2011). This appears plausible as the loss of the arolium occurs frequently in insects (Beutel & Gorb, 2001, 2006) and there is no apparent reason why tipulomorph dipterans should have evolved (or re-activated) this structure. Moreover, the secondary absence in the potentially basal Nymphomyiidae and Deuterophlebiidae can be easily explained with their specific biology (short lifespan, adults submerged (at least temporarily), miniaturisation).

Arolia are widely spread within Acercaria and a groundplan feature of this lineage (if it is monophyletic). They are almost generally present in Auchenorrhyncha (with the exception of

Cicadidae), in Coleorrhyncha, and in Thysanoptera. An arolium is also present in males of the genus *Corixidea* and males of several other members of Schizopteridae (Dipsocoromorpha; Emsley, 1969; p. 20). In these cases it is clearly used for attachment during copulation. The females of Dipsocoromorpha lack any specific adhesive structures. The distinctly bilobed arolium of membracids and leafhoppers (Membracidae and Cicadellidae, Study II) has apparently evolved independently from the bilobed pad in Psylloidea (Sternorrhyncha), as potential autapomorphies of these lineages, respectively. A unique and apparently autapomorphic feature is the eversible, balloon-like arolium of Thysanoptera. In its retracted position it is enclosed between two valves. This is not the case in fulgorids and ceropids, where the arolium is also eversible to a certain degree (Frantsevich et al., 2008; Study II). As mentioned above, the arolium was considered a potential autapomorphy of Neoptera by Beutel and Gorb (2001, 2006). However, whether the unpaired pretarsal attachment pads occurring in different insect lineages are homologous is questionable. The sclerotization of the arolium of sawflies (Gladun, 2008) for instance, is completely different from the pattern found in the arolia of members of Cicadomorpha. But obviously attachment devices are highly variable and differences do not automatically imply non-homology. Due to the uncertain homology assessment (sclerites, internal structures etc.), it is not possible to decide whether the differences between arolia occurring in acercarian and holometabolan lineages are due to secondary modifications of substructures, or to non-homology of the unpaired pretarsal pads as a whole. In contrast, there is little doubt that the unmodified arolium of cercopoids and the bilobed arolium in membracids are homologous structures. This is clearly indicated by specific conformities in the inner structure and sclerotization of these devices (Study II). In both cases, the cuticle is thickened and composed of branched chitinous rods.

The cuticle of the contact zone is generally distinctly thickened in insects with arolia (Fulgoromorpha: Frantsevich et al., 2008; Blattodea: Roth & Willis, 1952; Orthoptera: Slifer, 1950; Mantophasmatodea: Eberhard et al., 2009; Phasmatodea: Scholz, 2009; Hymenoptera: Federle et al., 2001). It is formed by chitinous rods in different attachment devices (e.g., Beutel & Gorb 2001), but the arrangement of these structural elements varies. In Orthoptera the pads function as a damper in the context of jumping and landing (Goodwyn et al. 2006). The rods stabilize the shape of the pad to prevent strong deformation. A similar function can be assumed for Auchenorrhyncha which are almost generally characterized by a good jumping capacity (e.g., *Cercopis vulnerata*: Gorb, 2004; *Philaenus spumarius*: Burrows et al., 2006; *Cicadella viridis*: Bonsignori et al., 2012; Fulgoromorpha: O´Brien, 2002). Interestingly, the only non-jumping species of our taxon sampling within Auchenorrhyncha lacks the arolium, which suggests a possible correlation between these features. The architecture of the rods

may also help to adapt to irregularities of the substrate surface. A second layer of chitinous rods was described in this thesis for the first time. This additional layer is present in the investigated members of Cercopoidea and Membracoidea, but not in Fulgoromorpha. It probably increases the elasticity of the pad and is a potential apomorphy of Cicadomorpha.

A gland is present within the arolium in all examined members of Auchenorrhyncha and in *Tipula* (Study II and III, respectively). A gland directly located within the arolium has been only described for Mantophasmatodea (Eberhard et al., 2009) so far. However, it is conceivable that it is more common than previously thought. In cockroaches (Roth & Willis, 1952) and grasshoppers (Slifer, 1950) gland-like tissue is visible in histological sections of the pretarsus, but it was referred to as epidermis in these studies. The function of the gland is not entirely clear. The secretion probably plays a role in adhesion to smooth surfaces (e.g., Orivel et al., 2001; Jiao et al., 2000). However, in some species of Hymenoptera the secretion is not released externally (Billen, 1986, Jarau et al., 2005), but remains within the pretarsus. In Hymenoptera the gland is located in the distal tarsal segment, not the arolium itself. A hydraulic function has been proposed by Federle et al. (2001). The liquid from the gland reservoir is pumped into the arolium, thus resulting in partial unfolding to increase the contact surface with the substrate.

4.1.3 Pulvilli

Smooth pulvilli have evolved at least three times independently within Acercaria — in "Psocoptera", in a clade comprising aphids and scale insects, and in the "higher" Heteroptera. Interestingly, pretarsal or tarsal adhesive pads are absent in most of the presumably basal groups of Heteroptera, in Enicocephalomorpha, the semi-aquatic and aquatic lineages Gerromorpha and Nepomorpha, and also in Saldidae (Leptopodomorpha). Saldids are predaceous ground- dwelling bugs, and arguably the sister-group of the remaining terrestrial heteropteran lineages (Wheeler et al., 1993). A novel type of attachment device occurs in the megadiverse heteropteran subgroup Pentatomomorpha: paired pretarsal pulvilli. The presence of these adhesive structures is arguably related to a close association with plants. Pulvilli are also present in aphids and coccoids, and in "Psocoptera", apparently a result of parallel evolution and also related to phytophagous habits.

The shape of pulvilli can be very variable. This is conspicuous in the family Miridae (Schuh, 1976) and in Diptera. However, the characteristic thickened cuticle with chitinous rods found in arolia does not occur in pulvilli as far as known at present.

Smooth pulvilli are only present in two genera of the examined dipterans, in *Simulium* and *Androprosopa*. They are similar to those occurring in fleas (Beutel & Gorb, 2001) but the phylogeny clearly suggests that they have evolved independently, as these taxa are deeply

nested within lower dipteran lineages.

Hairy pulvilli are present in Bibionomorpha, Culicomorpha and most Brachycera. They are very likely secondarily missing in several groups. The shapes of pulvilli differ considerably across the Diptera. In Brachycera, for instance, the ventral surface of the pad-like pulvilli is covered with hundreds of capitate acanthae. In some species the pulvilli are not pad-like, but merely a bundle of acanthae. Aside from Diptera, pulvilli occur also in Siphonaptera (smooth), Trichoptera (smooth) and some Lepidoptera (hairy). They usually function as attachment devices in these groups. However, in Lepidoptera their assumed function is to protect the arolium from abrasion on rough substrates (Al Bitar et al., 2010).

4.1.4 Empodia

Hairy empodia are a groundplan feature and potential autapomorphy of Diptera. Their presence is very often linked with the presence of pulvilli. Their shape is just as variable as the shape of pulvilli. A well-developed pad-like empodium with its ventral surface covered with tenent acanthae is arguably a groundplan feature of Diptera. However, this interpretation implies complete reduction in Tipulomorpha (and secondary replacement with an arolium), and also secondary loss in *Mischoderus* and *Edwardsina*. The two genera *Tabanus* and *Pachygaster*, which are presumably close to the basal node of Braychycera, are the only members of this lineage with well-developed, pad-like empodia and pulvilli. In the "higher" Brachycera there is a trend towards reduction of the empodial pad. It is present as a long spine in the examined "higher" brachycerans (*Stilpnogaster*, *Episyrphus* and *Glossina*). A connection between the different types and the lifestyle of these taxa is not apparent. It is noteworthy that an arolium never occurs in combination with an empodium. It was suggested that the arolium and the hairy median lobe (mediolobus) of lower dipterans and lower brachycerans are homologous structures, based on a similar origin from the area distad the unguitractor plate (Stuckenberg, 2001; Sinclair & Cumming, 2006). However, considering the completely different ventral surface of the two structures this interpretation appears unlikely. The attachment devices in the basal family Deuterophlebiidae are characterized by a very pronounced sexual dimorphism. The very large empodium of male deuterophlebiids is very likely used during copulation in flight (Courtney, 1991), a specialized function different from the usual purpose of walking efficiently on different substrates (Beutel & Gorb, 2001). Males of *Deuterophlebia* are unable to walk in terrestrial environments (Courtney, 1991). They usually live close to streams, and the pad may also be used to break free from the stream surface and resume flight (Courtney, 1990).

4.1.5 Euplantulae

Among the examined groups smooth euplantulae occur only within Phthiraptera. No pretarsal attachment structures occur in this group. The complete reduction of the arolium is probably related to ectoparasitic habits and a potential autapomophy of true lice. Within the group, different tarsal and tibial devices have evolved. A specific type of euplantulae is present in amblycerans, especially in species specialized on birds as hosts. Interestingly, euplantulae on the proximal tarsomere are also present in species of the genus *Paraheterodoxus* (Boopidae, Marshall, 2003), even though these amblycerans do not live on birds, but in the fur of the rufous rat-kangaroo. This is an example of attachment devices of the same type adapted to very different environments. In Ischnocera and Amblycera the mandibles appear to play a more important role as grasping devices than specialized structures on the legs (Bush et al., 2006). In anoplurans the thumb-like process on the distal part of the tibia and the opposing claw enclose the hair. It is plausible to assume that the grasping mechanism is enhanced by the tarsal euplantulae. The same function is conceivable for the thick hyaline cones on the apex of the tibiae in the ischnocerans examined. These cones on the tibial apex are apparently typical for many species specialized on birds (Smith, 2001).

4.2 Phylogeny of Acercaria

The monophyly of Acercaria is well supported by the morphological data set presented in Study I. Psocodea, Thysanoptera, and Hemiptera share a set of synapomorphic features of different body regions, such as a chisel- or stylet-like lacinia, arguably a preadaptation for specialized sucking–piercing feeding habits, an inflated anterior region of the 2nd axillary sclerite, an extremely compacted abdominal ganglionic chain, and a reduced number of Malpighian tubules (shared with Holometabola excl. Hymenoptera; Beutel et al., 2011). The results do not support a placement of Zoraptera as the sistergroup of Acercaria (e.g., Hennig, 1969; Beutel & Weide, 2005). The precise position of this small and enigmatic order is not settled yet. However, there is an increasing consensus that they should be placed among the polyneopteran lineages (e.g., Kukalova-Peck and Peck, 1993; Wheeler et al., 2001; Yoshizawa & Johnson, 2005; Yoshizawa, 2007; Ishiwata et al., 2011; Yoshizawa, 2011; see also Trautwein et al., 2012). The placement of Acercaria as sistergroup of Holometabola is widely accepted even though poorly supported by morphological data (e.g., loss of larval ocelli; e.g., Beutel & Gorb, 2001, 2006). A clade including Acercaria and Holometabola (Eumetabola) is also tentatively supported by our data (with a very limited holometabolan taxon sampling) and by molecular studies (e.g., Kjer, 2004; Ishiwata et al., 2011). However, this requires further confirmation. A concept of paraphyletic Acercaria with Psocodea as

sistergroup of Holometabola as shown in Ishiwata et al. (2011) appears unlikely considering the morphological evidence. However, recent results of the 1KITE project support the same arrangement based on analyses of transcriptomes of hexapod terminal taxa representing all orders (unpublished results, pers. comm. B. Misof).

4.2.1 Psocodea

A clade Psocodea is well supported by unique morphological features (Rudolph & Knülle, 1982; Seeger, 1975) as well as molecular data (Murrell & Barker, 2005; Cryan & Urban, 2012; Ishiwata et al., 2011). A highly unusual apomorphic groundplan feature identified by Seeger (1975) is the cibarial water-uptake apparatus. An additional presumptive groundplan apomorphy is the mortar-and-pestle apparatus of the cibarium. It is still retained in the groundplan of Phthiraptera but reduced in the majority of its subgroups (e.g., Tröster, 1990). The relationships within Psocodea are still not fully clarified. However, a sistergroup relationship between Liposcelididae and true lice seems to be well supported by morphological characters such as enlarged hindfemora and fused pterothoracic nota (Lyal, 1985), and also by analyses of molecular data (Yoshizawa & Johnson, 2003; 12S, 16S rDNA). This renders "Psocoptera" paraphyletic. Phthiraptera (true lice) were weakly supported as a monophyletic unit in our analyses. Potential apomorphies are the reduced number of antennal flagellomeres, a condition also occuring in Heteroptera and Coleorrhyncha, and the simplified ovipositor (well developed in the psocodean groundplan). Moreover, the complete reduction of the flight organs (absent or distinctly reduced in Liposcelididae), a dorsoventrally flattened body (also in Liposcelididae), and ectoparasitic habits were considered as obvious candidates for phthirapteran autapomorphies (see e.g., Grimaldi & Engel, 2005). Despite this seemingly strong morphological evidence, the monophyly of the true lice was questioned with respect to Amblycera in recent studies based on molecular data. Analyses of 18S rDNA (Johnson et al., 2004; Murrell & Barker, 2005) yielded a clade Amblycera + Liposcelididae, and the remaining true lice as its sistergroup. This hypothesis suggests that parasitism in this lineage has evolved twice independently and also a series of features characterizing the four ectoparasitic groups. However, the results of Murrell and Barker (2005) also include the unlikely paraphyly of Hemiptera and an unorthodox placement of Coleorrhyncha as sistergroup of Auchenorrhyncha. Considering the morphological data and the specialized ectoparasitism on mammals and birds, a clade Phthiraptera seems more likely, but further confirmation by more extensive molecular data is required.

4.2.2 Condylognatha

The placement of Thysanoptera is a matter of longstanding controversy (e.g., Kristensen et

al., 1991). The morphological characters we analysed support a clade Condylognatha, i.e. a sistergroup relationship between Thysanoptera and Hemiptera. Potential synapomorphies include the stylet-like mandibles (right mandible vestigial in thrips), a specifically articulated distal median plate of the forewing, the reduction of the maxillary palps (absent in Hemiptera, fewer than four segments in thrips), and a dorsal shift of the anterior tentorial pits. The same result was obtained in several studies using different morphological character sets (e.g., Kristensen, 1981; Yoshizawa & Saigusa, 2001; Wheeler et al., 2001, fig. 10; see also Hennig, 1969) and was also tentatively supported by molecular data analysed by Ishiwata et al. (2011). The alternative hypothesis, a clade Micracercaria (Thysanoptera + Psocodea), is suggested by the presence of an enlarged dorsal cibarial muscle with an unpaired median tendon (e.g., Willmann & Dathe, 2005). A sistergroup relationship between Thysanoptera and Psocodea was also tentatively supported by analyses of 18S rDNA and 28S rDNA (Wheeler et al., 2001) and a study using seven gene regions (Cryan & Urban, 2012). As in the study of Ishiwata et al. (2011), the sampling of psocodeans and thrips was very limited in Cryan and Urban's (2012) study, which focused on hemipteran relationships. The results of Wheeler et al. (2001) have to be taken with caution. Neither the analyses of 18S rRNA nor those of 28S rRNA sequences (Wheeler et al., 2001: figs 13 and 14) supported a clade only containing the psocodean and thysanopteran terminals.

4.2.3 Hemiptera

There is no doubt about the monophyly of Hemiptera (Auchenorrhyncha, Heteropterodea, and Sternorrhyncha; e.g., Hennig, 1969; Kristensen, 1981; Kristensen et al., 1991). The most conspicuous autapomorphy is the characteristic labial rostrum, with completely reduced palps and endite lobes. This finding is clearly supported by our own data and also by molecular studies using different data sets and analytical approaches (Kjer, 2006; Ishiwata et al., 2011; Cryan & Urban, 2012). Our data turned out to be insufficient for resolving the interrelationships of the three hemipteran subgroups. Schuh (1979) suggested Sternorrhyncha as the sistergroup of the remaining three lineages, thus rendering "Homoptera" paraphyletic. The same conclusion was reached by Popov (1981, palaeontological data), Zrzavy (1992, morphological and ecological data) and Cryan and Urban (2012, extensive molecular data). A taxon consisting of Heteropterodea (= Prosorrhyncha) and Auchenorrhyncha was referred to as Euhemiptera. This was also supported in several studies analysing different partial sequences of 18S rDNA (Campbell et al., 1995; Dohlen & Moran, 1995; Sorensen et al., 1995).

4.2.4 Auchenorrhyncha

A clade Auchenorrhyncha was well supported by our data. The presence of a complex tymbal acoustic system appears as a convincing argument for this clade. Within the group, Fulgoromorpha were also clearly confirmed as a monophyletic unit. The monophyly of Auchenorrhyncha was also supported by analyses of sequences of a broad array of genes (Urban & Cryan, 2007; 18S rDNA, 28S rDNA, Histone 3, Wingless) and in an even more extensive study using seven gene regions (Cryan & Urban, 2012; 18S rDNA, 28S rDNA, histone H3, histone 2A, wingless, cytochrome c oxidase I, NADH dehydrogenase subunit 4). Auchenorrhyncha was challenged as a clade in other studies. A sistergroup relationship between Cicadomorpha and Aphidoidea was suggested based on characters of the head capsule by Hamilton (1981), and a closer relationship between fulgorids and true bugs was proposed by von Dohlen and Moran (1995). The latter study was only based on 18S rRNA, and the taxon sampling was very limited, with only nine species of Auchenorrhyncha included. A sistergroup relationship between Cicadomorpha and Heteropterodea (Heteroptera + Coleorrhyncha) appears as a serious alternative to the monophyly of Auchenorrhyncha. This was suggested in an evaluation of combined paleontological, molecular, and morphological data (Bourgoin & Campbell, 2002) and also supported by a recent study based on transcriptomes (1KITE, Letsch et al., 2012). Considering the conflicting hypotheses, the issue of the monophyly of Auchenorrhyncha should be considered an unsolved question.

4.2.5 Heteropterodea (= Prosorrhyncha, Coleorrhyncha + Heteroptera)

Coleorrhyncha are clearly placed as the sistergroup to Heteroptera. The same result was supported by Cryan & Urban (2012) and in other studies (Wheeler et al., 1993; Ouvrard et al., 2000; see also Schlee, 1969), and also by a recent detailed comparative study of head structures (Spangenberg et al., 2013). Furthermore, the wing-coupling structure of moss bugs is very similar to that of heteropterans (D'Urso, 1993). The monophyly of Heteroptera was clearly confirmed, whereas the relationships within the group remained unresolved. This is mostly due to the very fragmentary knowledge of the morphology of supposedly basal groups such as Enicocephalomorpha and Dipsocoromorpha (Wheeler et al., 1993; Xie et al., 2008; summarized by Weirauch & Schuh, 2011). The basal branching events in Heteroptera are not fully clarified yet. A recent analysis of multiple genes yielded a basal position of Nepomorpha (Li et al., 2012).

4.2.6 Sternorrhyncha

Sternorrhyncha were clearly confirmed as a clade. Autapomorphies are the posterior shift of the proboscis between the procoxal bases and the membranous posterior parts of the

head capsule. A sistergroup relationship between Psyllidae and Aleyrodidae (Psyllomorpha) was also well supported, mostly by characters taken from Schlee (1969), who proposed this hypothesis earlier. Synapomorphies are the ductus ejaculatorius modified as a sperm pump, the constriction of the abdominal base, the broad and closely adjacent hind coxae, and the pedunculate eggs. In several studies based on 18S rDNA (Campbell et al., 1995; Sorensen et al., 1995), Psyllidae were placed as the sistergroup of the remaining Sternorrhyncha, and Aleyrodidae as the sistergroup of a clade comprising of Aphidoidea and scale insects. The same relationships were inferred from DNA nucleotid sequence data from seven gene regions (Cryan & Urban, 2012). Whiteflies share at least some morphological features with aphids and scale insects, such as a reduced wing venation, sedentary or sessile nymphs, and antennae reduced to six or fewer segments (e.g., Grimaldi & Engel, 2005). Apparently the interrelationships of the sternorrhynchan subgroups require further investigation.

4.3 Interactions of plant surfaces and attachment structures

The presence of wax blooms on plant surfaces provides protection against insect herbivores by impeding their attachment for feeding or oviposition (e.g., Brennan et al., 2001, Bodnaryk, 1992, White & Eigenbrode, 2000). The presence of deterrent epicuticular lipids (Powell et al., 1999) and glandular trichomes (Alvarez et al., 2006) has been shown to affect aphid host selection. In this thesis plant-insect interactions were investigated using the example of the pea aphid (Study IV). The species *Acyrthosiphon pisum* (pea aphid) encompasses genetically distinct sympatric host races. Each host race shows a preference for a certain legume species. The leaflet surfaces of these plants differ considerably in their wax coverage. Three host races were used to assess whether aphids show differences in their attachment ability on the respective host and non-host plants with different surface topologies.

Traction forces were generally lower on plant surfaces with wax blooms. The substrate probably contaminates the attachment structures, thus lowering the attachment ability of the insects distinctly (Rutledge & Eigenbrode, 2003; Gorb et al., 2008). Additionally, the presence of wax blooms increases the microscopic surface roughness, which in itself is sufficient to reduce insect attachment (Scholz et al., 2010). Scholz et al. (2010) showed that surface roughness within scales of a specific length prevents adhesion by creating a pattern too rough for adhesive pads but not rough enough for claws.

On *Vicia faba*, however, which is considered the universal host plant for all pea aphid races, the attachment forces were even slightly higher than on the reference substrate (glass plate). On this plant wax platelets that might have an influence on the attachment ability are missing. The leaflets showed the highest density of stomata among the examined plants. These

stomata increase the roughness of the surface and possibly provide additional purchase for the insects. The presence of trichomes on the plant surface can aid the locomotion of the aphids. During the experiments we observed the insects trying to hold onto the trichomes with their claws, thus creating leverage to pull themselves forward.

The presence of wax platelets on the surface of the leaflets of the different plants also influences the subsequent performance. Aphids put onto a glass plate immediately after walking on the respective leaflets had problems where they had performed well before. This reduced attachment ability is another indicator for the aforementioned contamination of the attachment pads. After walking on *Vicia*, the only examined plant without wax platelets, the subsequent attachment ability was not affected. This contamination effect is only temporary. After walking a couple of centimeters the aphids were able to produce the previous traction forces before contamination. This suggests that they are able to remove the wax from their adhesive organs. It has been shown that adhesive pads are able to "self-clean" with repeated steps (Clemente et al., 2010). With each step they leave parts of the particles on the surface. A similar procedure is conceivable in aphids, since the attained traction forces increase with each step, even though the aphids do not show any active grooming behavior.

The assumption that aphids might perform better on their respective host plant was not confirmed. The aphid varieties did not perform better on their hosts compared to non-host plants. This suggests that they are not better adapted to walking on their host plants, and that chemical plant factors within the plant tissues (e.g., Schwartzkopf et al., 2013) are more important in the context of host plant choice than the plant surface structure. It has to be taken into consideration that aphids do not feed on the leaflets alone. Especially nymphs are often found on the stems and tendrils where attachment is easier.

Even though these results suggest that the attachment ability on plant surfaces does not play a role in maintaining the host races, further research on this issue is necessary. As the wax blooms can also reduce the attachment capacity of parasitoids and predators of aphids, it can influence the regulation of aphid populations by their natural enemies. Populations of predaceous coccinellids for instance did not differ consistently between reduced-waxbloom versus normal-waxbloom peas, failing to support a hypothesis that predator populations are more dense on reduced waxbloom peas (White & Eigenbrode, 2000).

4.4 Conclusions and outlook

Even though this thesis made a contribution towards solving the relationships within Acercaria, some issues could not be sufficiently clarified. The monophyly of Auchenorrhyncha, for instance, is supported by numerous morphological characters, but challenged by

molecular studies. The results of most molecular studies are not consistent, depending on which genes and/or taxa were studied. This makes it difficult to develop evolutionary scenarios for attachment structures. The inclusion of more genes and a broad taxon sampling are necessary to yield more robust results. This might be achieved in the near future by the 1KITE project (see above) or the i5k initiative (sequencing of the genomes of 5000 insects and other arthropods). Considering the enormous amount of data (and therefore characters) in recent molecular projects, morphological characters will only play a minor role in reconstructing phylogenies in the future. However, they are still essential as an independent source of information for testing results based on molecular data (and vice versa). Moreover, they are essential for tracing the evolution of the groups in question on the phenotypic level. Last but not least they are the only source for placing fossil taxa, and thus essential for understanding the evolution of any groups in the dimension of time (Beutel et al., 2011).

As pointed out by Beutel and Gorb (2001), attachment devices can provide phylogenetic information despite obvious functional constraints. Additional apomorphies of adhesive devices strengthened the support for different lineages (Psocodea, Fulgoromorpha, Heteroptera). Linked with a remarkable versatility in habitat choice, a very wide spectrum of attachment devices has evolved in Acercaria, with the notable exception of hairy pretarsal adhesive pads. Tracing the precise evolutionary pathways of dipteran adhesive devices is apparently a challenge, even though some phylogenetic interpretations appear straightforward, such as the presence of a distinctly developed empodium as a derived groundplan feature of Diptera, and hairy pad-like pulvilli as a groundplan autapomorphy of a clade including Psychodomorpha, Bibionomorpha, Culicomorpha and Brachycera. What remains problematical is the connection between the general morphology and different life habits and preferred substrates on the one hand, and the specific adhesive devices on the other. There is no apparent configuration of attachment structures linked to either feeding on nectar or honeydew or sucking blood of vertebrates. Size reduction may have affected the complexity of attachment structures in some cases (e.g., Drosophilidae) but apparently not in others (e.g., *Mayetiola*, *Pachygaster*). In contrast to some specialized phytophagous insects in other groups, for instance beetles (e.g.,, Beutel & Gorb, 2001), dipterans seem to be less specific with respect to the substrates they attach to and walk on.

The character system is also affected by phylogenetic constraints. The type of attachment device within a family is always constant, even if the species live in distinctly different habitats. Individuals of the genus *Paraheterodoxus*, for instance, have the same attachment devices (euplantulae) as the rest of ambylcerans, even though they live on mammals not on birds. This is an example of how attachment devices of the same type can function on or

adapt to different surfaces or structures.

Attachment structures are affected by a high degree of homoplasy. Several structures (e.g., pulvilli, tarsal attachment pads) evolved several times independently within Hexapoda. There is also a very high variability concerning the morphology of the devices (e.g., shape, sclerotisation, size and number of tenant hairs). Some of these characters do not depend on the lifestyle or habitat, but for instance on body mass. Gorb et al. (2001) showed that the setal tip area and setal density depend on this parameter. The capacity to adapt the adhesive devices to different surfaces and functional requirements is probably one factor that has contributed to the extreme diversification of insects, especially in groups closely associated with plants.

Clearly defined major evolutionary trends are not easily recognized. The arolium is a groundplan feature of Neoptera, but was reduced several times independently in different lineages. All adhesive devices are prone to abrasion when used on rough substrates. A trend towards inflatable or movable arolia is possibly related to this phenomenon, with modified adhesive pads coming only in contact with the surface if needed (e.g., Mantophasmatodea, "heelwalkers", arolium bent upwards during normal walking; Thysanoptera, arolium covered by protective valves; Hymenoptera and Diptera, movable arolia). This reduces the wear and tear of the devices. There also seems to be a trend towards hairy attachment structures. Hairy pulvilli and empodia are common features in holometabolous insects. Among the non-holometabolous groups they occur only in Dermaptera, Embioptera and a single family of Heteroptera (fossula spongiosa in Reduviidae). The adhesive hairs in Dermaptera are not homologous with the ones in the examined dipterans. In the latter group they are microtrichiae, not setae.

Plants evolved effective mechanical and chemical deterrents against insect herbivores. The presence of wax blooms in particular reduces the attachment ability. In the case of pea aphids the attachment ability does not play a role in the maintenance of the host races, and therefore speciation. However, it is unclear if the presence of wax blooms has the same effect on the typical predators of aphids, which exhibit entirely different types of attachment devices. Therefore, further research on the attachment ability of these predators (e.g., hover flies, lady bugs) is necessary.

5 Summary

Aims of the present thesis were (1) a detailed study of the attachment structures of carefully selected taxa, (2) the reconstruction of the phylogeny of Acercaria based on a morphological data set containing characters of all body parts including adhesive devices, (3) the creation of evolutionary scenarios for attachment structures, and (4) the investigation of interactions between plant surfaces and attachment devices.

Attachment devices of 59 (excl. outgroups) species were described and illustrated, including key taxa like Coleorrhyncha, Enicocephalomorpha, Dipsocoromorpha (Hemiptera, Acercaria), or Deuterophlebiidae (Diptera) (Study I, II, III). Attachment devices can provide phylogenetic information. However, they are not suitable for resolving entire phylogenies without additional data, as they are affected by a high degree of homoplasy.

In the case of Diptera evolutionary changes were interpreted based on recently published phylogenies. Combining features of attachment structures with data from other studies, a phylogenetic analysis was conducted with 118 characters scored for selected terminals of all major acercarian subunits. The results support the monophyly of the entire lineage and the major subgroups Psocodea, Phthiraptera and Hemiptera. “Psocoptera” were rendered paraphyletic. The data also supported the monophyly of Auchenorrhyncha and a sistergroup relationship between Thysanoptera and Hemiptera (=Condylognatha).

The arolium is a groundplan feature of Neoptera (with several independent secondary losses), whereas other devices (pulvilli, euplantulae) evolved several times independently in different lineages. There is a trend towards movable and inflatable arolia, which only come into contact with the surface if needed. There is also a trend towards hairy adhesive structures, which are a common feature of holometabolan insects.

The traditional definition of arolium was expanded and refined. It includes the internal morphology, and therefore structures with previously uncertain homology could be clearly classified (e.g., bilobed arolia of Membracoidea). A gland situated within the lumen of the arolium was described for the first time for Auchenorrhyncha (Study II).

The interactions between insects and plant surfaces were studied using the example of the pea aphid complex (Study IV). The presence of wax blooms reduces the attachment ability of insects permanently. Pea aphid varieties did not perform better on their native host plants compared to non-host plants. Therefore it seems that the attachment capacity does not play a role in the maintenance of the host races, and therefore speciation. Further research is necessary to understand the intricate relationships between attachment devices and surfaces.

6 Zusammenfassung

Die vorliegende Arbeit hatte folgende Ziele: (1) Die detaillierte Beschreibung der Morphologie von Haftstrukturen ausgewählter Taxa, (2) die Rekonstruktion der Verwandtschaftsverhältnisse der Acercaria basierend auf Merkmalen aller Körperteile einschließlich Haftorganen, (3) die Erstellung evolutiver Szenarien für Haftstrukturen, sowie (4) die Untersuchung der Interaktionen zwischen Haftorganen und Pflanzenoberflächen.
Die Haftstrukturen von 59 Insektenarten (zuzüglich 18 Außengruppenvertreter) wurden im Detail untersucht (Publikationen I, II, III). Das Taxon Sampling beinhaltete auch sogenannte Schlüsseltaxa, wie Coleorrhyncha, Enicocephalomorpha, Dipsocoromorpha (Hemiptera, Acercaria) und Deuterophlebiidae (Diptera). Haftstrukturen können zwar phylogenetische Information liefern, allerdings sind sie nicht hinreichend um die Phylogenie und Evolution einer Großgruppe zu rekonstruieren, da sie sehr häufig konvergent entstanden sind.
Bei den Diptera wurden die evolutiven Veränderungen anhand kürzlich publizierter Systematiken interpretiert. Für Acercaria wurde eine cladistische Analyse durchgeführt, die 118 Merkmale aller Körperteile und Vertreter aller Ordnungen beinhaltete. Die Ergebnisse dieser Analyse stützen sowohl die Monophylie der Großgruppe selbst, als auch die Monophylie der Ordnungen Psocodea, Phthiraptera und Hemiptera. „Psocoptera“ hingegen erwiesen sich als paraphyletisch. Desweiteren wurden die Monophylie der Auchenorrhyncha und ein Schwesterngruppenverhältnis von Thysanoptera und Hemiptera (=Condylognatha) unterstützt.
Das Arolium ist bereits im Grundmuster der Neoptera vorhanden, wurde aber mehrfach sekundär reduziert. Andere Haftstrukturen hingegen, wie Pulvilli oder Euplantulae, evolvierten mehrfach konvergent zueinander.
Ein evolutiver Trend geht in Richtung eines beweglichen und entfaltbaren Arolium, welches nur in Kontakt mit dem Substrat kommt, wenn es nötig ist. Es gibt ebenfalls einen Trend hin zu haarigen Haftstrukturen, welche ein sehr häufiges Merkmal der holometabolen Insekten darstellen.
Die traditionelle Definition des Aroliums wurde erweitert und verfeinert. Die innere Morphologie wird jetzt mitberücksichtigt; dadurch konnten Strukturen mit bisher unklarer Homologie eindeutig zugeordnet werden. Eine Drüse, die sich direkt im Lumen des Aroliums befindet, wurde hier erstmalig für die Auchenorrhyncha beschrieben (Publikation II).
Die Interaktionen zwischen Haftstrukturen und Pflanzenoberflächen wurden am Beispiel des Erbsenlaus-Komplexes untersucht (Publikation IV). Die Verschmutzung der Tarsen durch Wachskristalle auf der Pflanzenoberfläche verringert die Haftfähigkeit von Insekten drastisch. Die verschiedenen Blattlaus-Varietäten konnten sich im Vergleich nicht besser

auf ihren jeweiligen Wirtspflanzen fortbewegen als auf Nicht-Wirtspflanzen. Im Fall der Erbsenblattlaus scheint die Haftfähigkeit demnach keine Rolle bei der Aufrechterhaltung der Wirtsrassen, und damit bei der Speziation, zu spielen. Weitere Forschung ist nötig, um einen besseren Einblick in das komplexe Wechselspiel zwischen Haftorganen und Oberflächen zu bekommen.

7 References

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8 Curriculum vitae

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10/2005-06/2010	Diplomstudium Biologie Abschlussprädikat „sehr gut“ (1,3) Thema der Diplomarbeit: “Die Kopfmorphologie von *Phyllium siccifolium* (Insecta: Phasmatodea) und ihre Bedeutung für die Phylogenie der Euphasmatodea“
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9 Eigene Publikationen

Friedemann K, Beutel RG. (in press) Morphology of arolia in Auchenorrhyncha (Insecta, Hemiptera). *Journal of Morphology*, DOI 10.1002/jmor.20290

Friedemann K, Schneeberg K, Beutel RG. (2014) Fly on the wall - attachment structures in lower Diptera. *Systematic Entomology* 39, 460-473.

Friedemann K, Spangenberg R, Yoshizawa K, Beutel RG. (2014) Evolution of attachment structures in the highly diverse Acercaria (Hexapoda). *Cladistics* 30, 170-201.

Spangenberg R, **Friedemann K**, Weirauch C, Beutel RG. (2013) The head morphology of the potentially basal heteropteran lineages Enicocephalomorpha and Dipsocoromorpha (Heteroptera, Hemiptera). *Arthropod Systematics and Phylogeny* 71, 103-136.

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Friedemann K, Wipfler B, Bradler S, Beutel RG. (2012) On the head morphology of *Phyllium* and the phylogenetic relationships of Phasmatodea (Insecta). *Acta Zoologica* 93, 184-199.

Konferenzbeiträge

2010, 2011	Graduiertenforum der Fachgruppe Morphologie der Deutschen Zoologischen Gesellschaft (DZG)
2011, 2012, 2013	International Max Planck Research School (IMPRS) Symposium
2012	Graduiertentreffen der Fachgruppe Zoologische Systematik der Deutschen Zoologischen Gesellschaft
2013	6th Dresden Meeting on Insect Phylogeny

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11 Eigenständigkeitserklärung

Hiermit erkläre ich, Katrin Friedemann, geboren am 15.07.1986 in Karl-Marx-Stadt, dass ich die hier vorliegende Dissertation „The evolution of attachment structures in two megadiverse insect lineages - Acercaria and Diptera“ selbstständig und nur mit den darin angegebenen Hilfsmitteln verfasst und an keiner anderen Fakultät oder Universität eingereicht habe. Ich erkläre außerdem, dass ich bisher keine Promotionsversuche unternommen habe. Darüber hinaus ist mir die Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena bekannt. Für die Anfertigung dieser Arbeit habe ich weder die Hilfe eines Promotionsberaters in Anspruch genommen, noch haben Dritte unmittelbar oder mittelbar geldwerte Leistungen von mir erhalten, die im Zusammenhang mit der hier vorliegenden Dissertation stehen. An der Erstellung der in der vorliegenden Arbeit verwendeten Originalarbeiten / Manuskripte haben Co-Autoren mitgewirkt. Mein Eigenanteil ist für jede Arbeit einzeln angegeben.

Katrin Friedemann Ort, Datum